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THE EARLY AMERICAN NATURALIST AS REVEALED BY LETTERS TO THE FOUNDERS¹

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On November 22, 1866, F. W. Putnam, A. S. Packard, Jr., Alpheus Hyatt, and E. S. Morse, curators at the Essex Institute in Salem, Massachusetts, drew up their plans for the *American Naturalist*. These four young naturalists, who until a short time before had been students of Louis Agassiz, launched a publication which has continued to the present day. While it has been eminently successful in many respects, it has undergone periods, of great difficulty and uncertainty, especially in the early days of its history. L. C. Dunn (1944), the present editor of the journal, and E. G. Conklin (1944) have traced some of the highlights in the founding and early history of the *American Naturalist*. Other historical accounts will be found in the biography of E. S. Morse by Wayman (1942) and in the history of the Peabody Museum of Salem by Whitehill (1949).

At the Peabody Museum of Salem, which was organized as the Peabody Academy of Science by these same men at about the same time the journal was founded, there are twenty-six bound volumes of letters sent to the founders of the *American Naturalist* during the first few years of its existence. These have been studied by the writer, and selections have been made from this vast amount of correspondence which illustrate some of the problems the founders faced and the reaction of readers to the journal. These excerpts, together with letters possessed by the founders' families, form the basis for the present report, which portrays the early *American Naturalist* as seen by correspondents.

¹Grateful acknowledgment is made to Ernest Stanley Dodge, Director of the Peabody Museum, Salem, Massachusetts, for permission to study records on file in that institution and for an early photograph of the museum; to Miss Alice Putnam for the privilege of reading her father's papers deposited in the Archives of Harvard University and for the loan of a portrait of Frederic Putnam; to Mrs. Frances Packard McClellan for the loan of her father's diary and a portrait of A. S. Packard; to Mrs. Harriet Hyatt Mayor for loan of letters belonging to her father, Alpheus Hyatt; and to Dr. L. C. Dunn for the photographic reproductions of the title page and table of contents of the first issue of the *American Naturalist*.

In organizing the journal F. W. Putnam was placed in charge of the printing since he founded and owned the Salem Press which printed this pioneering magazine. A. S. Packard had charge of literary matters and did most of the editing over a long period of time. Alpheus Hyatt was in charge of the business affairs and kept the books, while E. S. Morse took charge of subscriptions and mailings. He also prepared many of the illustrations, being one of the most skillful scientific artists of his day. W. S. West was hired to help with the clerical work and Caleb Cooke, a staff member at the museum, also assisted in the routine tasks of issuing this publication.

The American Naturalist was the first illustrated popular magazine of science produced in this country. In the first three volumes all four men are listed as editors. In volumes four and five Packard and Putnam are named as editors, while Morse and Hyatt are listed as associate editors. The following four volumes record Packard and Putnam as editors with R. H. Ward as associate editor in charge of the Department of Microscopy. Beginning with volume ten the journal was published by H. O. Houghton and Company of Boston, but Packard continued as its editor. It was published continuously until 1878 under the auspices of the founders, after which time it passed into other hands. The founders, however, continued to give it support and made contributions to its pages over a long period of time.

In the first issue the young naturalists stated their purpose and aspirations as follows: "We shall endeavor to meet the wants of all lovers of nature—shall popularize the best results of scientific study and thus serve as a medium between the teacher and student. Shall it do no more than to bring naturalists, both young and old, into an active co-operation and sympathy, and promote good fellowship and amity between the great brotherhood of enthusiasts, as all true naturalists are, we shall gain a most important object.... We trust the Magazine will be equally welcome to the farmer, gardener and artisan. We shall endeavor to point out the practical benefits resulting from the study of nature."

Packard wrote enthusiastically to his father upon returning from a business trip to New York on Mar. 17, 1867, as follows: "I was on the go all day and did heaps of business for the *Naturalist* and learned much of the art of magazing, to coin a word, and of the methods of pushing the magazine with the market. I was glad to find on my return that we have already sold over 1,000 copies! Shall have to print 2,000 of number 2. It is going like hot cakes!" At the end of the second year of publication, 2,500 copies of each issue were printed at a cost of \$500. The actual circulation at that time was about 2,100. Packard's father, a professor at Bowdoin College, wrote to his son on Mar. 11, 1868, "I think your last number of the *Naturalist* an excellent one. Your own articles always read very well."

The journal was well received by both scientists and laymen. A review in the *Quarterly Journal of Science* (London) stated that, "The leading men of science in America are among its contributors, and it is in every way worthy of the great nation which it is intended to interest and instruct."



FIGURE 1. Frederic W. Putnam, publisher of the early *American Naturalist*.

C. H. Hitchcock wrote on May 15, 1867, "My dear Packard.—I first saw in Paris a prospectus of your *Naturalist* and a copy of the first number at the house of Mrs. Marcou. I admire your courage and perseverance in attempting such an arduous work and wish you great success." Prof. Marcou, himself, later wrote that, "C'est une bonne et utile publication." Theodore Lyman wrote on June 19, 1867, "Prof. Agassiz directs me to acknowledge the receipt of numbers 1, 2, 3, 4 of your very valuable and handsome publication sent for the library of the museum and to return you his sincere thanks for the same." Among the lay readers such comments as the following were received: From Brownsville, Pa., Aug. 16, 1867, "A friend has kindly sent me some specimen numbers of your magazine. It is *just the thing* that I have been looking for these five years past. It shall not die for want of my support. I enclose \$3.00 for which please send to my address the *Naturalist* for 1869." Another letter from Brooklyn, N. Y., Feb. 1, 1869, reads, "I enclose \$4.00 for the *Naturalist* this coming year. The work is not only valuable to the general reader, but to the student and scholar as well—and is in every respect a credit to the Institute from which it emanates. I hope during the present year to visit the Peabody Institute. It seems to have more life and genuine enthusiasm among its workers than any other similar one in the country." Lt. Charles Bendire, U. S. Army, wrote from Fort Lapwai, Idaho Territory, on Jan. 10, 1869, "Looking through an old file of papers of the year '67, I saw a brief article on the first appearance of the *Naturalist*. I have been wanting to become a subscriber of a paper or a magazine of this sort published in the United States. Please send me a number, if possible the first issued in '69, as a sign of the *Naturalist's* existence and I shall remit the subscription price for the year with pleasure, by return mail."

The magazine seemed to fill admirably the need for which it was intended. A letter from Dayton, Ohio, August 21, 1867, reads, "I am much pleased with your magazine. It is just such a magazine as the student of natural history has long since wanted, and I hope you will meet with all the encouragement you need." Another one from Syracuse, N. Y., July, 1867, says, "Some such publications shoot over the heads of common people and are food only for those far advanced. The mass needs reliable plain common-sense relations and descriptions from keen-eyed observers. Let the common and scientific twins go hand in hand so that he that reads may read and understand what he reads and learn where to look and how to look and how to be benefited himself or interest others in what he may discover." For some subscribers the magazine served as an outlet for those in search of serious literature. A letter from Monticello, Iowa, Dec., 1868 gives this point of view, "The study of natural history needs to be encouraged among the people. If it could take the place of much of the trashy literature of the day it would do more to educate them and to support their wants and better satiate their mental needs." And again from Greencastle, Ohio, Feb. 19, 1869, "As I wish to make natural history my special study and not having the



FIGURE 2. A. S. Packard, Jr., principal editor of the early *American Naturalist*.

means to purchase those large and valuable works on that subject, I shall rely upon you and your valuable magazine for various instructions."

A good deal of correspondence was received concerning contributions from naturalists of the day who had apparently been solicited for papers. Samples of this type of correspondence follow. From F. V. Hayden (Director of Survey of the Territories) Omaha, Neb., Aug. 20, 1867, "I will send several articles on Indians with wood-cut illustrations—3 or 4 each.... I wish you would notice the Geological Survey of Nebraska in your journal. Success to you in your laudable efforts." Then from Colorado on Nov. 20, 1867, Hayden wrote, "My dear Packard, I will do all I can to write you some articles but I am driven almost to death."

Joseph Henry, Secretary of the Smithsonian Institution wrote on Nov. 4, 1868, "We enclose some interesting articles on the *Habits of the Burrowing Owl* and the *Shedding of the Horns of the Antelope* (the former by Dr. Canfield, the latter by Mr. Hurman) which you are at liberty to publish in the *American Naturalist* giving proper credit to the Smithsonian Institution." He wrote again on Dec. 11, 1869, "Herewith we enclose the manuscript of an article on *The Sea Otter* by Capt. Scammon of the U. S. Revenue Service in order that it may be published in the *Naturalist* if you desire it for that purpose."

A. E. Verrill wrote from New Haven, Conn., Nov. 5, 1868, "I hope to write an article soon giving a sketch of our dredging trip and noting some of our more interesting discoveries." And another one from New Haven written by O. C. Marsh dated Nov. 27, 1868, "My dear Hyatt, Your note requesting a review of Dana was duly received. As I have just returned from another western trip, I am particularly busy and so not to disappoint you, I have asked Prof. Banker to write it."

Trenton, N. J., "I send you today, my first opportunity, the article on fish of which I had made mention. I have spent much time over it culling the sweets of all 2,000 pages of notes taken down at odd times not far apart since the summer of 1855." Charles C. Abbott.

New Haven, Conn., May 10, 1869, "I am sorry to say, in reply to your instruction to write an article for the *Naturalist* on the crabs of our coast, that my time is so occupied that it will be impossible for me to do anything of the kind this term—Mr. Verrill has written to you this evening and has also begun his article for the *Naturalist*." S. I. Smith. Chicago Academy of Science, Nov. 3, 1869, "My dear Packard, I returned the proof yesterday—I can't promise about the Florida paper. My time day and evening is about entirely taken up by Academy duties for I have to do everything that requires any knowledge beyond that of an ordinary clerk—I envy you Salem fellows and often wish I could get together such a coterie of savants here." William Stimpson.

From the letters of Edward D. Cope the following are quoted. Hall of the American Museum of Science, Jan. 15, 1869, "I send a rough sketch of a plate I want cut in wood to illustrate the *American Naturalist* if you will have it. It will accompany part two of the *Fossil Reptiles of New Jersey*

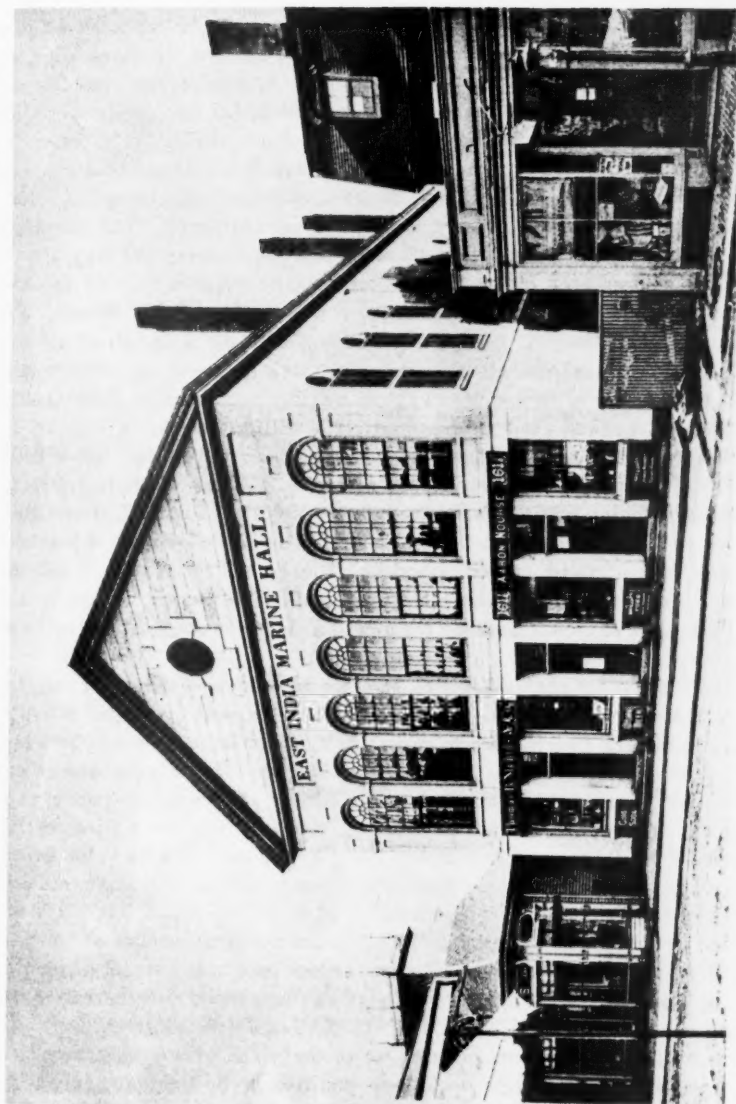


FIGURE 3. Peabody Academy of Science in Salem at which the *American Naturalist* was founded. Photo taken about 1870.

which will occupy eleven pages or so of the *American Naturalist*. . . . I hope that whoever draws it on wood will replace the dark lines by fine lines and shade, etc., with a true artist's eye. This any good man can do: I have not time for more than the animals and the scene. Cannot your cutter be the one who did Wood's *Phalangiidae*? The work ought to be at least as good as that done for the illustrated papers, which our scientific cuts usually are not." Philadelphia, June 13, 1869, "I hope to fulfill my promise to send you an essay on whales one of these days." Haddonfield, N. J., March 7, 1870, "I will write in a few days the desired review of Leidy's book."

Cambridge, Mass., Jan. 19, 1869, "I presume there would be no difficulty about the wood cuts certainly not as far as I am concerned. You can have them no doubt by applying to S. J. Scudder in whose possession they are. I am rather hard pressed for the present but have no doubt to be able to send you something before many weeks for the *Naturalist*." Jeffries Wyman. Ft. Macon, N. C., March 26, 1870, "I had rather intended a sketch of all our sandpipers but found the article was getting too long and so left off at a good stopping place. If you have no more eligible matter for the July number I could work the rest into a separate article." Elliott Coues.

February 5, 1869, "I am glad that the manuscript was of use and will do the same for you again when leisure offers." William H. Dall. Later he wrote, Smithsonian Institution, April 4, 1870, "My dear Packard, Better late than never and I enclose a short article for you as I promised and if you like it, I will follow it with others by and by. Please tell Hyatt that I think the Chicago Academy will illustrate the Polyzoa, at all events he had better make drawings of them. Give my regards to Putnam, Morse, and the rest and believe me. Ever truly yours, William H. Dall."

In addition to the professional naturalists who were solicited for articles many amateur scientists sent voluntary contributions. Typical of such letters is the one from Mackerel Corner, N. H., Dec. 1869, "I send you a few items from my notebook and if these notes are acceptable will send others—I do not write from but what I know and have seen with my own eyes." From the Smithsonian Institution, Apr. 22, 1869, "I enclose an interesting article—*The Arkansas Fly Catcher*—which you may like to print in the *Naturalist*. It was written by Robert Ridgeway, one of our new ornithologists and of much promise." Spencer F. Baird.

From Fort Paul, Minn., Oct. 3, 1868, "I have a great amount of information in regard to the surface geology and some important general principles derived from them which I would be glad to furnish you with hereafter if I can get time from my official duties." G. K. Warren (U. S. Army).

Whenever there was any question as to the value of any voluntary contribution, advice was sought from those qualified in the field concerned. We find such letters as the following. Cambridge, Sept. 26, 1868, "You asked if certain notes of William P. Bolles are worth publishing. I should hesitate, though that is what half the English local literature consists of—new localities and observations upon the most trivial variations." Horace Mann. And again from Horace Mann on Oct. 4, 1868, "I beg you will not advertise

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POPULAR ILLUSTRATED MAGAZINE
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VOLUME I.



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FIGURE 4. Title page of the first issue of the *American Naturalist*.

or sell that catalogue of Canadian Plants by Hubbert. He is dead, to be sure, but it is one of the most miserable and inadequate things ever printed being a compilation from old botanies which do not begin to give the thing as it is correctly." A comment from J. A. Allen of Cambridge reads, "Jan. 13, 1870, Dear Putnam, The notes sent a day or two since in respect to our winter ducks are interesting and important. Professional naturalists rarely have the opportunity of becoming so well acquainted with this class of birds as sportsmen do."

A good deal of correspondence was received by the editors requesting information of all kinds pertaining to natural history. Some of these letters, with the answers, were published in the *Naturalist*. Others were answered privately. Samples of this type of correspondence follow. July 5, 1867, "Will you please inform me what to understand in the following, found on page 273 *American Naturalist* seventh line from top 'Man had a common origin.' When reading it yesterday I could not take from it a satisfactory sense, and I trust you will oblige me by gratifying my curiosity to understand what I read." Grand Isle, Vt., Oct. 8, 1867, "Enclosed you will find some specimens of land snails which I found in this vicinity. I hope to learn their names and send them to you for that purpose. Will you give them in your next issue of the *Naturalist*." Sept. 18, 1867, "I cannot find any definition of *slide*. Even in Webster's large dictionary, such as I presume is meant. Since giving my attention to readings in natural history I have met with many words not to be found in the more common dictionaries." Nov. 8, 1868, "Can't you give me a recipe in the next *Naturalist* for a mucilage or glue with which to affix numbers and other labels to minerals and which will resist both damage and dry atmosphere?" Nov. 24, 1868, "Can you give me information in relation to the so-called *monster* on exhibit in Boston at corner of Union and Hanover streets? Is it a humbug like Barnum's mermaid or a monstrosity or a distinct species of shark or its allies?—I questioned the exhibitor in relation to the teeth, bones, food, etc., of the beast but found his accounts somewhat conflicting."

The intention on the part of the founders to have the *Naturalist* serve as a source of information on economic entomology seems to have met a real need during the early years of publication. Some of the correspondence concerning these matters is extracted below. April 15, 1867, "Every farmer in Kansas would be greatly profited this season to understand the natural history of the red-legged locust which covered the country last August and September destroying all the land crops... I consider the *Naturalist* a necessity to the farmer, especially the Kansas farmer." Baltimore, May 20, 1867, "Will you not permit me to suggest that you publish in your excellent journal common sense articles on *Cimex lectularius* (bedbug) and *Musca domestica* (housefly)." Tehama, Calif., May 30, 1867, "I particularly desire to see the articles on the American silkworm as silk culture is attracting much attention in California. Our soil and climate appear to be significantly adapted to that enterprise. From present appearances it is not impossible that in twenty years silk growing will be the leading interest of California."

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FIGURE 5. Partial Table of Contents of the first volume of the *American Naturalist*.

Rockford, Ill., July 26, 1867, "If you haven't already, I hope you will give us something on the potato bug—which is committing great ravages entirely destroying whole patches of potatoes."

The early magazine was also intended to stimulate youth in the study of natural history. A letter from the mother of Bryant Walker, who later became a leader in American Conchology, reads: "Detroit, Mich., Sept. 11, 1867, Enclosed you will find \$3 for the present year's issue of your magazine of which I have seen a number which has highly interested me and delighted my son Bryant Walker, a lad of eleven years of age who is enamored of the study of natural history and is already conversant with Audubon and Wilson's ornithology. He is taking lessons in taxidermy, etc." A letter from Kennebunk, Maine, Nov. 11, 1867, "Mr. Morse, I have been reading the *American Naturalist* and was very pleased by the descriptions given by you of the land snails of New England, and I am going to try to get a collection—I am only thirteen years old but I like the study of natural history better than anything else." Oberlin, Ohio, Feb. 24, 1868, "I have only been taking your magazine but one year and I am well pleased with it. I am but a boy and very fond of the study of nature, and you may look for my subscription at the close of each volume." One lad wrote, "I am very much interested in the study of nature and expect to become a naturalist. If you who are specialized would write an article entitled 'Letter to a Young Naturalist' it would, I think, just suit many who read your magazine." Another wrote from Winchendon, Mass., Aug. 19, 1869, "Enclosed is a manuscript which, if it possesses sufficient merit, I should like to see in the columns of the *American Naturalist*. In case it should meet with your approval, I will become an occasional contributor to your timely, valuable magazine. As an apology for the mistakes which you may possibly detect in the accompanying manuscript, I would state that I am but sixteen years old, and although I have written for the press, I have never before written an article for a strictly scientific journal. Will you please write me your honest opinion of the article and oblige." W. J. Beal wrote from Chicago, Ill., Dec. 27, 1869, "I am doing what I can in and about Chicago to raise up a race of naturalists."

From the very beginning the *Naturalist* had many financial difficulties. John Baldwin Coolidge wrote from Lawrence, Sept., 1867, "I am very sorry to learn that the magazine is not paying its way... I will do all in my power to help you." Among the reasons for the financial problems which arose were the numerous requests for free subscriptions and special discounts. Unfortunately a great many people who were most interested in the magazine found the cost of the subscription to be a burden if it could be afforded at all. Among those making a request for free copies or reduced subscription rates we find the following. July 1, 1867, "Do you make any discount for clergymen and teachers?" March 12, 1869, "As president of the Reading Public Library I wish to know if you make any different terms with librarians from the advertised ones. The town of Reading is poor in money but exceedingly rich in wants." Oberlin, Ohio, Sept. 18, 1869, "As

treasurer of the Reading Room Association of this place, I write to you to ascertain the price per year of the *Naturalist*. We have been able thus far to secure some deduction from the regular prices of the different periodicals. I wish you might send me a specimen copy for the committee to examine and state the lowest possible terms to which it can be had." East Boston, Mass., Jan. 11, 1870, "Is it asking too much to request you to donate your publication to our association (Y.M.C.A.)?" Sept. 3, 1869, "I am anxious to become a subscriber to the *American Naturalist* but I am unable to do so for want of funds. Can I pay for it from volume one onward in any objects of natural history in the region which you may desire?" A correspondent from Ohio sums up the situation which has been unfortunately too often true: Oct. 7, 1867, "Everywhere I find a taste for light reading and so little interest taken in anything of a scientific nature that the people seem quite unwilling to pay for it unless they can get it cheap."

Other than financial, the main problems were concerned with improper mailing and billing of the magazine. Many of these were undoubtedly attributable to the fact that there were too many persons concerned with the publishing of the magazine. Often times, one of the four was not aware of what some of the others were doing which lead to frequent confusion. Also there were occasional clerical errors of mailing and keeping records which gave rise to complaints on the part of subscribers. The following examples will illustrate some of these problems. Waddington, N. Y., Aug. 7, 1867, "I have written several letters about my July number of the *Naturalist* being missing but you do not seem to understand me for I am still getting August numbers—have three now. I should like a July number very much to fill my set." Otter Creek, Ill., July 6, 1867, "A short time ago I sent \$3 for a years' subscription to the *Naturalist* and yesterday a package of four came to hand containing a receipt—when lo!! and behold!!! they were all alike—the March number—which was most too much of a good thing seeing I had had a copy sometime ago and read it several times." San Francisco. Sept., 1867, "Unless packages are sent by steamer they will not be likely to reach this office. Large quantities of mail matter lie on the overland route and is used to fill up holes beside cannons." Exeter, N. Y., May 10, 1869, "Where is my *Naturalist*? I hear that my article is out and have been wanting to see how it looks so as to judge about writing another. J. W. Chickering, Jr." New York, Sept. 19, 1867, "There appears to be a 'screw loose' in your *American Naturalist* affairs. I have just received a third bill for the *Naturalist* for 1867 with the request that I remit 'early' the sum of \$3. I find I have a receipted bill for this signed 'A. Hyatt' and dated April 3, 1867." Chicago, Ill., Sept. 23, 1867, "For the third time I am favored with your bill for this year's magazine. Twice have I stated that I have paid." Academy of Natural Science, Philadelphia, Dec. 21, 1869, "I returned to you today the last number of the *Naturalist* addressed by you to Dr. Leidy, it being imperfect. Please send another number to the Academy as soon as possible."

Complaints from authors were usually concerned with editorial changes or alterations which displeased the original writer. We find, for example, letters such as the following. "Newtonville, Mass., Sept., 1869, I regret to find that although I expressed my regrets in the proof of my article on the 'Supposed New Columbine and New Ox-eye' that the names of Miss Grace H. Crockett and Mrs. Alida C. Avery had been omitted as the discoverers of the daisy. The article appears without the names. This omission may seriously compromise me here among the students and officers and I regret exceedingly that you should find it necessary to depart from the ms. for there is a pride felt about the discovery." North Madison, Conn., Feb. 24, 1869, "You did not consider the notes on the breeding habits of a few of our birds which I sent you some months since worth publishing. I may be egotistical in thinking them as interesting as some which have since been published." W. Newton, Mass., Aug. 12, 1869, "I have just read in the August number of your magazine the communication I sent you on the Tennessee Warbler and am displeased at two changes you have taken the liberty to make (unless I am very much mistaken) from the original."

Much of the correspondence from authors concerned the preparation of illustrations. Two typical ones are given here. Office of the State Entomologist, St. Louis, Dec. 29, 1868, "Friend Packard, I don't know when your plate on which you figure the moth I sent will be cut but my lithographic figure will be in a short time and will perhaps have the priority. Therefore, if I adopt your name I want *your own* description.... I would like you to send me the complete technical description which you have drawn up for your own use." C. V. Riley. From New Haven, Conn., March 4, 1870, "My dear Packard, I find on examination that I have not got a decent proof of the head of *Branchipus* although the negative is good. So in regard to the *Artemiae* and *Branchinecta*—the proof which I enclose is by no means as good as we can get—We had better wait a few days until Smith (Sidney I. Smith) can get a chance to make some more proofs. Perhaps it would be well to engrave one of the heads of *Branchinecta* or perhaps its egg case in order to make the paper more complete." A. E. Verrill.

Many requests were received for publicity. Some of these were commercial and others of professional interest. Manufacturers of scientific apparatus wrote such offers as "I will send you a microscope (the Craig microscope) prepaid with some mounted microscopic objects if you will notice it editorially in the *American Naturalist*." Professional notices were requested such as the one from C. H. Hitchcock from Hanover, N. H., Feb. 25, 1869, "If you choose to note briefly the fact of an organization of a Geological Survey of New Hampshire, I shall not object." Offers for exchange notices were numerous. One from the *Maryland Educational Journal* read, "May 20, 1867. I am willing to give you good notices in return for the same on your part towards this journal in as much as we too (to quote your words) also 'need every encouragement in starting our enterprise'." From the University of Michigan, Ann Arbor, Jan. 20, 1869, "We have sent you a copy of our University Magazine hoping to be able to place your publication on our ex-

change list. We do not expect you will receive an equivalent for your publication in our magazine, but we give extended notices of our best exchanges."

Zoology has always been the main interest of the *Naturalist* although in the early years there was a section on botany and one on microscopy. A letter from Asa Gray suggested that the *Naturalist* increase its service to botany. It read in part, "Botanic Gardens, Cambridge, Mass., Feb. 14, 1870, I am telling our scattered botanists they should make the *Naturalist* their organ of communication, and I think it would be to your good to enlist botanists more. There is a plan in Illinois to extend the *American Entomologist* to include botany. I tell them better support one journal well, such as yours, than have more and feeble ones. If you will take to botany I will endeavor with Mr. Hughes' help to aid you."

Several letters show a prevailing interest in the work of Charles Darwin, whose *Origin of Species* had only recently been published. A surprising letter was received by Dr. Packard from C. H. Hitchcock, writing of his trip to England, dated May 15, 1867, as follows: "I also attended 80 lectures on zoology by Professor Huxley who is truly a live practical naturalist not carried away by Darwinism enough to injure the integrity of his researches. He presented both sides of the development question more fairly than it has been done by any Progressivist I have met—so much so indeed that tho' I had a leaning towards Darwinism his facts have cured me of it."

About that same time a paper on the American Deer with evolutionary bearing was published by J. D. Caton in the *Transactions of Ottawa Academy of Natural Science*. This stimulated a good deal of comment in the pages of the *American Naturalist*. The author sent the following note to the editor with a copy of his letter from Darwin. Ottawa, Ill., Dec. 11, 1868. "I take the liberty of enclosing a copy of a letter I have received from Mr. Darwin on my paper on the 'American Cervus'.—Sept. 18, 1868, Dear Sir: I beg leave to thank you very sincerely for your kindness in sending me through Mr. Walsh your admirable paper on the American Deer. It is quite full of most interesting observations, stated with the greatest clearness. I have seldom read a paper with more interest—for it abounds with facts of direct use for my work. Many of them consist of little points which hardly anyone besides yourself have observed or perceived the importance of recording. I would instance the age at which the horns are developed (a point on which I have lately been in vain searching for information) the development of horn on the female Elk and especially to different natures of the plants consumed by the deer and elk and several other points. With cordial thanks for the pleasure and instruction which you have afforded me and with high respect for your powers of observation. I beg leave to remain, Dear Sir. Yours faithfully and obliged, Charles Darwin."

A relevant note received by the *Naturalist* from Asa Gray reads, Dec. 21, 1869, "The Spike-horn Deer and some other things in December number of your journal will interest Mr. Darwin. Could you mail to him the sheet of the Miscellany—to Down Beckenham, Kent? I oblige. Yours, Asa Gray."

While the journal was notably successful in accomplishing its stated aims and was conducted on a high professional level, it suffered from financial difficulties from the very beginning. In May of 1875, Putnam approached the trustees of the Essex Institute to shoulder the burden of the *Naturalist*. Receiving no encouragement from them, Putnam then approached Henry Holt to take it over. Holt wrote on June 30, 1875, that "The only safe general principle in these times appears to be to buy nothing, but my respect for the periodical would lead me to at least carefully consider any information you may see fit to give regarding its business condition and the requirements of its owners." A detailed answer sent by Putnam on July 5, 1875, summarizing the historical development and financial plight of the *Naturalist*, reads, "In answer to your note of June 30 about the *American Naturalist* I hardly know what to say except to give you a general statement as to how we have managed the magazine and the success we have had. The magazine was first started under the auspices of the Essex Institute by four of us who were unpaid officers of the Institute and with the simple and pure idea of doing missionary work in science. We did not look to making money out of the book but we did hope that in time we should make the magazine pay its expenses and enable us to use it for the benefit of our society. After about one year from the start Mr. Peabody gave the money to found the Peabody Academy of Science in Salem and we four editors of the magazine were made officers of the new Academy and put on salary. We, therefore, turned out the Magazine in the name of the Academy and made great use of it for the benefit of the Academy in many ways especially in sending it all over the world in exchange for the publications of other societies, journals, etc. In this way we had a large circulation, but as we never made special business of obtaining subscribers our subscription list was never one-quarter what it could have been made had we devoted time to it. All we could do with the scientific work that was on our hands was to edit the journal and let the subscribers come as they would, a poor way so far as money was concerned but a splendid thing for the reputation of the journal and to the credit of our Academy—then came these hard times when every business required to be well looked after and we find that we are out of pocket on the Magazine and our Trustees think that it will be outside of the duties of their trust to take the responsibilities of a business operation on their hands when their trust is for a specific purpose—we are forced to sell the Magazine for what we can get in order to have it continue in its good work which is of great object—if my letter is not a business one, inasmuch as it gives both sides of the story at once, you will the better be able to judge of the prospect of making the magazine a paying thing when taken up and managed on true business principles.—Now as to making the rate and the price. Parties who have looked into the matter have stated that we ought to get at least 10 or \$12,000 for all the property and good will and I feel that it is well worth that. In fact in 1873 we were offered 10,000 for it by a respectable house that has since taken up other matters. In these times of course we do not expect so

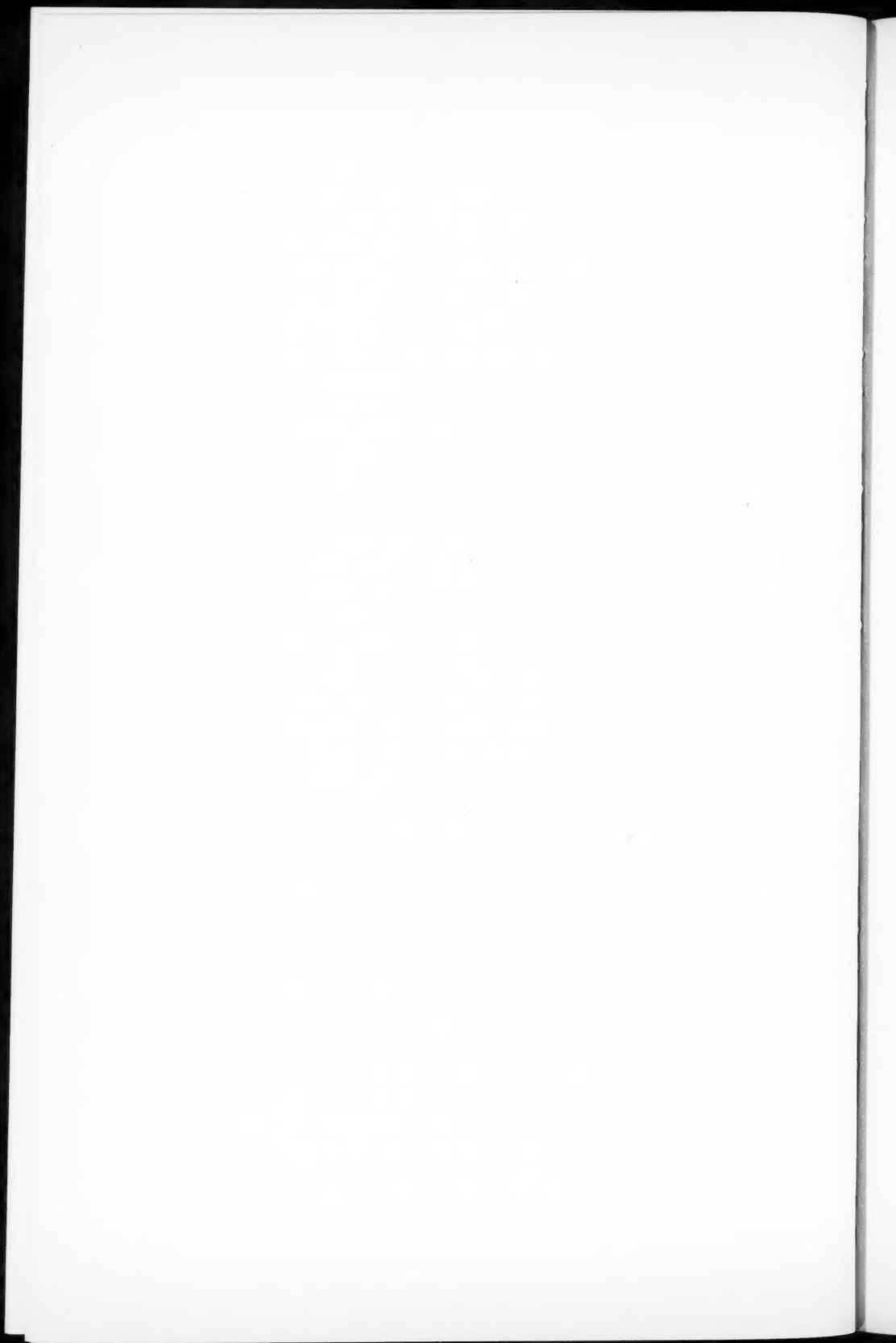
large a sum and we are open to bids below those figures.—Should you decide to purchase and take it off our hands at once, Dr. Packard and myself would agree to do the editorial work free for the rest of the year or until you make other arrangements and, if desired, Dr. Packard would take the position of editor at fair rates. My other duties have so much increased of late since my appointment in Cambridge that I should have to give up the editorial work with the Dec. number."

Twenty-two days later Holt wrote to Putnam, "I may as well tell you that I do really want to do this thing. The question is whether I can afford to." Five months later Holt wrote again, "In ordinary times I should be very glad to expand in the direction of science even so far as the commercially limited field of technicalities. But, this is no time to expand anywhere." Eventually H. O. Houghton and Company took over the publication of the journal. Dr. Packard received the following letter dated Oct. 26, 1875: "Referring to the conversation between you and our Mr. Houghton today we would say that we are willing to publish the *American Naturalist* for two years provided the gentlemen who are interested in its continuance will guarantee us against loss."

Thus, the *American Naturalist* left Salem. Only Packard, who was to continue to serve as chief editor for a period of ten years more, but who himself left Salem for Brown University in 1879, remained with the journal. After two years Houghton and Company passed the publication on to the press of McCalla and Staveland of Philadelphia. Packard and Edward D. Cope then served as joint editors. After volume twenty, none of the original founders continued in an official capacity.

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A STUDY ON THE NATURE OF AUTONOMY IN NEOPLASTIC PLANT CELLS*

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The tumor problem is a problem of growth and more particularly a problem of the growth of altered cells by more or less random division. The tumor cell is not, however, characterized primarily by its rate of growth since certain normal cell types may grow and divide at considerably faster rates than do most tumor cells. The tumor cell need not, moreover, be limited in a capacity to differentiate and functionate. Pluripotent teratoma cells possessing malignant properties are quite capable of showing a high degree of differentiation. The one biological character that distinguishes tumor cells from normal cells is the capacity for the uncontrolled or autonomous growth of the tumor cell within an organism. Without this there would be no tumors.

Autonomy of neoplastic growth has many gradations. At the one extreme are the so-called benign tumors which commonly grow slowly and remain localized in the host. At the other extreme are the most malignant cancers which invade neighboring tissue and spread throughout an organism by metastasis. The nature of autonomy remains one of the foremost problems in cancer research.

Since the capacity for autonomous growth is potentially a property of the cell itself, it should be equally capable of expression in the cells of all higher organisms whether they are of animal or plant origin. It does not necessarily follow, however, that the cellular mechanism, in terms of specific chemical reactions, is identical in members of the two kingdoms. The fundamental similarity of cells and cellular processes is, nevertheless, commonly recognized and advances in one field of investigation may assist in clarifying problems in related fields of study.

PROCESSES IN THE GENESIS OF TUMORS

One striking aspect of tumor genesis is the multiplicity of diverse processes that are seemingly capable of accomplishing the same end result. Radiant energy, hereditary factors, irritation, carcinogenic chemicals, microorganisms, and viruses have all been shown to be more or less effective as incitors of tumors in animals and plants. With the possible exception of the hereditary constitution of the host and of certain of the viruses, these factors are concerned with the inception of tumors and do not appear

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to be involved in the continued abnormal growth of tumor cells once the alteration of normal to tumor cell has been consummated. In an attempt to develop a concept designed to explain the action of the plurality of these diverse agents in the genesis of tumors, it has been postulated by some that these factors act as mutagenic agents and bring about the alteration of one or more genes in the affected cells. Others have suggested that the carcinogenic factors activate latent viruses present in certain cells. Still others have indicated that the uncontrolled growth of tumor cells may result from the selection or modification of certain normal cytoplasmic constituents which compete with or replace normal constituents concerned with the regulation of growth. Whatever the merits of these hypotheses, they do not give the desired information regarding the ultimate mechanism involved in the uncontrolled growth of cells within an organism. To state simply that a mutation has occurred or that a virus is responsible for tumor cell growth is in itself quite meaningless when attempting to characterize the underlying reason for autonomous growth. It is essential to define in physiological and biochemical terms the nature of the hypothetical mutation or to determine more precisely how the presence of a new self-duplicating entity in a cell affects the metabolism of a cell if an understanding of autonomy is to be achieved. It is with a consideration of this problem at a physiological level that we shall concern ourselves at this time.

CROWN GALL OF PLANTS

The experimental system that will be subjected to analysis is the neoplastic disease of plants known as crown gall. Crown gall is unique among diseases of bacterial origin in that the inciting bacterium is only indirectly involved in the causation of the disease. Susceptible and properly conditioned plant cells are regularly altered to tumor cells in short periods of time by means of an as yet uncharacterized tumor-inducing principle that is associated with the presence of the living bacterium. Once the alteration of normal cells to tumor cells has been fully accomplished, the role of the bacterium in the disease process is completed. The continued abnormal proliferation of the altered cells becomes completely independent of the inciting organism. When, for example, virulent bacteria are inoculated into susceptible plant species and allowed to act on the cells of the host for 72 hours before being destroyed selectively by thermal treatment, large rapidly growing tumors result (Braun, 1943, 1947a). When, on the other hand, the bacteria are killed after acting on the host cells for only 34 hours, small slowly growing tumors develop. Some of these overgrowths continue to grow very slowly, become encapsulated, and after prolonged incubation they may reach moderate size (Braun, 1951). Sterile tissue isolated from the slowly growing tumors grew very slowly in culture when compared with the rate of growth of cells isolated from rapidly growing tumors, the cells of which were acted upon by the bacteria throughout a 72-hour period. Since the characteristic growth levels of these two types of tumor cells are retained indefinitely in culture, it would appear that the degree of

cellular alteration attained in 34 hours is far less than that found when bacteria act on the host cells for a 72-hour period.

Slowly growing benign tumors can also be obtained by inoculating susceptible plant species with strains of the crown gall bacterium that show varying degrees of attenuation. The weakly virulent A66 strain initiates the formation of small slowly growing tumors on tomato (fig. 1,B). The highly virulent A6 strain, from which the attenuated A66 strain was derived (Hendrickson, *et al.*, 1934), regularly produces large rapidly growing tumors in that host (fig. 1,A). The T37 walnut strain is intermediate in its dis-

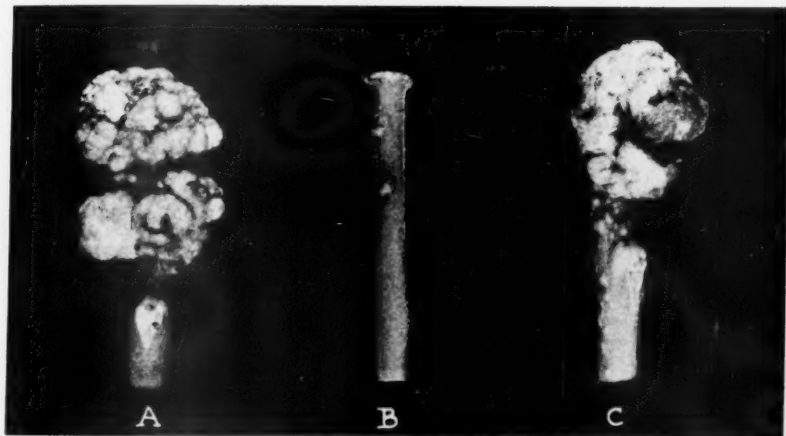


FIGURE 1. Tomato stem inoculated with: A. The highly virulent A6 strain of the crown gall bacterium. B. The attenuated A66 strain. C. The attenuated A66 culture and treated at the cut stem end with naphthalene acetic acid, a synthetic growth substance possessing auxin activity. (Photographs by J. A. Carlile)

ease-producing ability when inoculated into sunflower and certain other hosts. Sterile sunflower tumor tissue altered by the moderately virulent T37 strain grows more slowly in culture than do comparable cells transformed by a highly virulent strain (Braun, 1953). When, instead of inoculating a plant such as the sunflower, the moderately virulent T37 strain is introduced into tobacco, the cells of which possess a high degree of pluripotency, then in place of the characteristic unorganized crown gall tumor a complex overgrowth or teratoma results. The capacity for organization of such pluripotent tumor cells is retained indefinitely in culture. A highly virulent strain of the crown gall bacterium, on the other hand, induces the formation of typical rapidly growing unorganized tumors in tobacco. In this instance the cellular factors concerned with organization are overwhelmed as a result of the alteration of normal pluripotent cells to tumor cells. There exists in crown gall, then, a variety of growth patterns. These

range from slowly growing benign to rapidly growing potentially malignant tumors, and from completely unorganized to highly organized overgrowths. All have their counterparts in animal pathology. How do crown gall tumor cells achieve the capacity for autonomous growth and why do they show varying growth patterns?

GROWTH PATTERNS AND AUTONOMOUS GROWTH

Investigations carried out in a number of laboratories (Braun, 1947b; de Ropp, 1947a; Gautheret, 1947) during the past decade have suggested that uncontrolled cellular growth in crown gall is concerned with the production of greater than regulatory amounts by the tumor cell of a growth-promoting substance. Auxin synthesized by such cells had commonly been regarded as playing an etiological role in the continued abnormal development of those cells. There seems little doubt that crown gall tumor tissue is hyperauxinic (de Ropp, 1947b; Henderson and Bonner, 1952; Kulescha, 1949; Kulescha and Gautheret, 1948; Link and Eggers, 1941). However, it was by no means certain that the characteristic behavior of the crown gall tumor cell could be explained solely or even in large part on the basis of high auxin levels in those cells. Attempts were therefore made to learn whether, in addition to growth substances of the auxin type, non-auxinic factors capable of stimulating active cell proliferation are present in crown gall tumor cells (Braun and Naf, 1954; Steward, *et al.*, 1955). The method used by Braun and Naf (1954) for demonstrating such substances was similar to that used by other investigators (Steward and Caplin, 1951; Skoog, 1954) for demonstrating the presence of non-auxinic growth factors in certain normal tissues and in fluid endosperm of the coconut. Newcomb (1951) had shown that normal tobacco pith cells enlarge greatly but do not divide in the presence of growth substances of the auxin type. Cells of this tissue were therefore suitable for detecting the presence of non-auxinic growth factors capable of stimulating cell division in tumor tissue extracts. When tobacco pith tissue was isolated and planted on White's basic culture medium, the cells of the pith did not divide but remained quiescent indefinitely. When the basic medium was supplemented with naphthalene acetic acid, a synthetic growth substance possessing auxin activity, at a concentration of 1 mg/liter, the cells of the pith tissue enlarged greatly in size but they did not divide. Extracts of crown gall tissue that had been sterilized by filtration and added aseptically to the basic medium did not encourage either the enlargement or the division of the pith cells. When, however, both the auxin and filter-sterilized tumor extract were added to the basic medium in optimal concentrations, a very rapid growth by cell division of the pith cells resulted. A direct correlation existed within limits (0.005 mg - 1 mg NAA/liter) between the concentration of auxin in a culture medium containing a constant volume of tumor extract and the amount of growth by cell division that occurred in the pith tissue.

In the presence of a concentration of auxin equal to 0.5 mg NAA/liter in a culture medium containing 10 per cent tumor extract, a very active pro-

liferation of the pith cells resulted. The growth pattern of the tobacco pith tissue in this instance showed a striking morphological and histological resemblance to tobacco crown gall tumor tissue of the unorganized type grown on the basic medium. When the concentration of auxin was reduced in the tumor-extract-containing medium to a level equal to 0.01 mg NAA/liter, the pith tissue grew slowly and in an unorganized manner for about 4 weeks. Thereafter numerous more or less organized structures covered the surface of the overgrowth. Such tissues showed a resemblance to crown gall teratomata of tobacco. It has thus been possible to reproduce under controlled conditions growth patterns that resembled, superficially at least, the morphologically distinct types of crown gall tumors that have been described as occurring on tobacco. These artificially stimulated pith tissues were, however, self-limiting and when the externally supplied stimuli were removed, their growth promptly stopped. Crown gall tumor tissue is, on the other hand, autonomous and is itself capable of synthesizing growth factors needed for its continued abnormal proliferation.

EFFECTS OF GROWTH SUBSTANCES

It seems quite clear from studies of the type reported above that at least two growth factors, one an auxin which is concerned, for purposes of this discussion, with cell enlargement and the other which is concerned with cell division, are essential if growth by cell division is to occur in tobacco pith cells. A biologically active substance possessing cell-division-factor activity has recently been characterized chemically (Miller, *et al.*, 1955). The tobacco pith cells have apparently lost, as a result of their differentiation, the capacity to produce physiologically effective concentrations of the two growth substances. Because both growth-substance-synthesizing systems appear to have been blocked in pith cells, it was believed that experiments carried out with the use of such specialized cell types might give insight into the nature of the cellular alteration in crown gall (Braun, 1956). If, for example, only the auxin system is activated as a result of the transformation of normal cells to tumor cells, then the altered pith cells should enlarge greatly without, however, dividing. If, on the other hand, the cell-division-factor synthesizing system is activated without a corresponding activation of the auxin system, then neoplastic growth should not result because, as indicated above, the cell-division factor without auxin is ineffective in initiating growth by cell division in tobacco pith cells. Only if both growth-substance systems are activated simultaneously following the alteration of normal cells to tumor cells will a tumor develop.

When a small block of tobacco pith tissue was isolated, planted on the basic culture medium and inoculated with crown gall bacteria, no tumors were produced. It was not surprising to find that pith cells were not altered to tumor cells under the conditions of this experiment since our earlier studies had shown that cell division or the processes leading to cell division are essential if normal cells are to be altered to tumor cells by the

crown gall bacteria. Histological studies showed that cell division had not occurred at the point of inoculation or elsewhere in the pith tissue fragment. The problem then was to effect a normal wound-healing response in the pith tissue. When vascular tissue, which has been shown to be a source of both auxin and cell-division factor, was isolated with the pith tissue and the pith was inoculated with crown gall bacteria, no wound-healing reaction and no tumor formation resulted. It is known, however, that certain substances including the two growth factors of interest in this study may be translocated basipetally in the vascular region of a plant stem. When pieces of pith together with elements of the vascular system were isolated and, instead of being placed on the surface of the agar medium in their normal position as had been done in the previous experiment, were placed top-side down on the agar medium, the pith cells at the upper surface were stimulated to divide. Cell division in this instance resulted from the lateral diffusion into the pith of the two growth substances that had accumulated in the region of the vascular elements at the cut stem surface of what was originally the basal end of the tissue fragment. Inoculation of crown gall bacteria into such healing pith tissue resulted in the formation of typical crown gall tumors. Having thus found a method for inducing a normal wound-healing response in pith tissue, the above experiment was modified in such a way as to permit the vascular elements to remain in contact with the pith tissue only until the growth substances leading to cell division had diffused from the vascular region to the pith. This required about 3 days at 25°C. Thereafter the vascular tissue was removed and discarded and the pith fragments were inoculated with bacteria. Typical tumors were initiated in these experiments. The results of this study indicate that as a consequence of the alteration of normal tobacco pith cells to tumor cells, the affected cells achieve autonomy in two most essential directions. Prior to their alteration to tumor cells mature pith cells did not produce physiologically detectable amounts of either the growth factor concerned with cell enlargement or that concerned with cell division, while following their conversion to tumor cells both substances were produced. If both growth-substance-synthesizing systems had not been activated following the cellular alteration, growth by cell division and, hence, tumor formation would not have resulted in the test system used in these experiments. It nevertheless seems unlikely that the tumor-inducing principle in crown gall, which appears to be highly specific in its action, acts directly on the two biosynthetic pathways simultaneously by accomplishing either the removal of two normally occurring inhibitory systems or by effecting an increased synthesis or more efficient utilization of both growth substances simultaneously but independently by the tumor cell. A more likely explanation would appear to be that this tumor-inducing principle exerts its specific effect on one of the growth-substance systems resulting in the production of greater than regulatory amounts of that substance by the tumor cell. Concomitant with this alteration, a change is effected in the second system as a result of which the cell becomes autonomous for both growth factors.

RESPONSES OF OTHER CELLS

While normal tobacco pith cells require an externally supplied source of both the cell-enlargement and cell-division factor for growth by cell division, certain other plant cell types need only the addition of the cell-enlargement factor to the basic medium for their continued growth in culture. Such isolated cell types appear capable of synthesizing the cell-division factor. In these instances, in contrast with normal pith cells, the cell-division-factor synthesizing mechanism appears to be very lightly blocked or not blocked at all. Such cell types are readily converted to tumor cells by crown gall bacteria. This suggests that the mechanism concerned with production of cell enlargement factor is affected in some as yet unknown manner by the tumor-inducing principle, leading to an increased synthesis or decreased destruction of auxin by the cell. Since cell-division-factor synthesis appears to be normally functional in cells of this type, the presence of greater than regulatory amounts of auxin in the cells permits the continued unregulated growth of these cells. Earlier studies have demonstrated (Braun, 1953) and results similar to those reported above (Braun, 1956) have confirmed the fact that cell division or the processes leading to cell division are essential if normal cells are to be changed to tumor cells in crown gall. The normal wound-healing response may thus serve a dual function in the transformation process: 1) to make vulnerable to alteration the cellular system specifically affected by the tumor-inducing principle, and 2) to set into operation the normal processes of cell division which in the presence of greater than regulatory amounts of one of the growth factors (auxin) continue to be functional indefinitely.

STIMULATION OF SLOWLY GROWING TUMORS

If, as this work suggests, autonomy in crown gall is simply a question of the activation in tumor cells of two growth-substance systems that are more or less strongly blocked in normal cells, then it should be possible to increase the rate of growth of slowly growing benign tumors by supplementing them with either the cell-enlargement or cell-division factor or with a combination of both growth factors. Let us now consider again for a moment two of the slowly growing tumors described earlier in this discussion. When the normally small overgrowths initiated by the attenuated A66 culture on tomato are supplemented at a distance with any one of several growth substances possessing auxin activity, such tumors grow rapidly virulent strain (fig. 1, C) (Braun and Laskaris, 1942; Thomas and Riker, 1948). Tomato cells altered by highly virulent strains of bacteria apparently produce, as a result of their conversion to tumor cells, high levels of available auxin. This growth substance appears, on the other hand, to be regulated at a much lower level in cells altered by the attenuated strain. In this instance the limiting factor for rapid autonomous growth seems to be an auxin.

The second system in which slowly growing benign tumors were obtained was one in which a highly virulent culture of the crown gall bacterium was

allowed to act on the host cells for only 34 hours before being destroyed selectively by a thermal treatment. Tumor cells isolated from such overgrowths grew, it will be recalled, very slowly in culture when compared with the growth of cells altered by the same strain of the bacterium in a 72-hour period. When the basic culture medium on which the slowly growing tumors were planted was supplemented with either naphthalene acetic acid, a substance possessing auxin activity, or with the cell-division factor, the rate of growth of these tissues did not increase over that found when similar tissue was grown on the basic medium itself. A combination of auxin and of tumor extract containing the cell-division factor applied to the basic medium permitted some increase in the rate of growth of these tissues. Growth levels in this instance did not, however, approach that of cells altered by bacteria in a 3-day period grown on the basic medium. It is obvious from experiments of the type described above that neither the cell-enlargement nor the cell-division factor was by itself limiting for rapid growth. An additional factor or factors had therefore to be sought. Our recent studies, which are as yet preliminary, have shown that when the basic culture medium was supplemented with both yeast extract and naphthalene acetic acid, the rate of growth of the cells altered in 34 hours approximated that of cells altered by the bacteria in a 72-hour period. Neither the yeast extract nor the naphthalene acetic acid incorporated singly into the basic medium was effective in increasing the growth rate of this tissue. Since both of these factors were essential for rapid growth, the results suggest that these slowly growing tissues not only possess a requirement for some factor or factors present in the yeast extract but that the capacity for the production of available auxin in these cells was fixed at the time of their conversion to tumor cells at a level suboptimal for rapid autonomous growth. Normal plant cells altered to tumor cells in a 72-hour period are themselves capable of synthesizing optimal or near optimal concentrations of the cell division, cell enlargement, and yeast extract factors for their continued rapid and abnormal proliferation. Those altered to tumor cells in a 34-hour period, on the other hand, have had their requirements in terms of rapid growth only partially satisfied for the cell-enlargement and yeast extract factors. Thus the transition from the slowly growing benign to the rapidly growing potentially malignant crown gall tumor cell appears to be a gradual one involving quantitative rather than qualitative differences.

WOUND HEALING AND TUMOR INDUCTION

Of interest in this connection is the relationship between normal wound healing and tumor induction. An excellent correlation has been found to exist, as shown schematically in Figure 2, between the stage in the normal wound-healing cycle in which the alteration of normal cells to tumor cells is accomplished and the size and rate of growth of tumors that subsequently develop. These results, which are reported in detail elsewhere (Braun, 1952, 1954), demonstrate that a conditioning of the host cells resulting

from the stimulus of a wound is essential if normal cells are to be changed to tumor cells. This conditioning process takes place gradually reaching its maximum effect between the second and third days after wounding and declines again as normal cell division begins and wound healing progresses toward completion. If the host cells are not adequately conditioned, as appears to be the case in both the early and late stages of

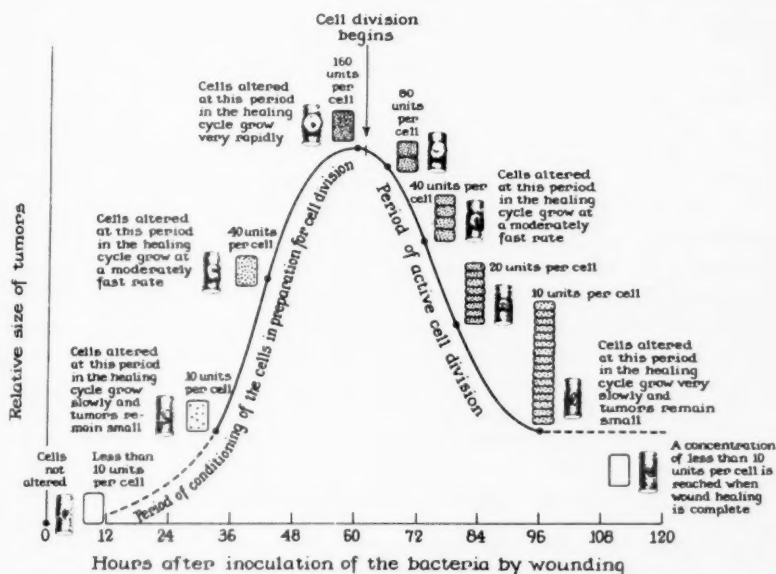


FIGURE 2. The relation of normal wound healing to tumor inception in crown gall. Conditioning of the host cells resulting from the stimulus of a wound is essential if the alteration of normal cell to tumor cell is to occur. The conditioning process, which is schematically represented, takes place gradually reaching a maximum between the second and third day after wounding and declining again as normal cell division begins and healing progresses toward completion. The size and rates of growth of the tumors produced can be correlated with the period in the normal healing cycle in which the cellular alteration is accomplished. (Photograph by J. A. Carlile)

wound healing as well as in most normal cells not under the influence of wounded tissue, then the cellular alteration will not occur despite the presence in the tissues of virulent bacteria. It thus appears that it is just before or during the earliest stages of active cell division in the normal wound-healing cycle that normal cells are most readily altered to tumor cells of the rapidly growing type. It is at this stage that the cells show a high rate of metabolic activity. Cells altered to slowly growing tumor cells in a 34-hour period are probably not fully conditioned and, hence, are not completely susceptible cells. As a result of this the cellular systems affected specifically by action of the tumor-inducing principle seem not to

be as vulnerable to modification 34 hours after wounding as they are at a somewhat later period, and, hence, only a partial alteration, leading to a slowly growing benign tumor, is achieved.

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STUDIES ON HOMEOSTASIS AND HETEROZYGOSITY I.
GENERAL CONSIDERATIONS. ABDOMINAL BRISTLE
NUMBER IN SECOND CHROMOSOME HOMOZYGOTES
OF *DROSOPHILA MELANOGASTER*.¹

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INTRODUCTION

The classical problem of evolutionary genetics has been for a very long time that of how a population adapts to an environment. More recently, students of evolution have come to realize that the real problem is not that of adaptation to *an* environment, but rather to the multiplicity of environments in which the individual and the species exists. In effect, the species must be not only a jack-, but master-of-all-trades, if it is to succeed in competition with other forms. As has been pointed out by Dobzhansky and Wallace (1953), Thoday (1953), Waddington (1953) and many others, this wide adaptation may be accomplished either by a polymorphism, whereby the species is a heterogeneous collection of genotypes each adapted to a specific environment, or else by individual adaptability such that each organism may itself adapt to a wide variety of environments. For most species these alternatives are combined in varying degree.

Accompanying this interest in the problem of diverse adaptation has been the appearance of diverse terminologies all meant to describe some aspect of the ability of organisms and groups of organisms to adapt to varying circumstances.

As this is the first of a projected series of papers dealing with this problem, it is essential to make some nomenclatural convention, to attempt to homologize it with ideas already current, and finally to remain consistent in its use.

DEFINITION OF HOMEOSTASIS

"Homeostasis" is a term which has been used by Dobzhansky and Wallace (1953) and by Lerner (1955) in their discussion of adaptation to diverse environments. As the title of this present paper indicates, it is this term which will be adopted for our discussion as well. In attempting to define homeostasis in a genetic context, it is best to look back to its original use by Cannon (1932). The sense of Cannon's "Wisdom of the Body" is that a homeostatic organism is one which maintains *certain aspects* of its physiology constant, despite environmental forces tending to disturb this constancy. The words "certain aspects" have been italicized

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because they lie at the very heart of the concept of homeostasis. Homeostasis does not mean constancy of all aspects of the organism's physiology. It does not mean constancy *per se*.

As an example one might consider the maintenance of body temperature in homoiotherms. In order to maintain temperature constant despite external fluctuation it is necessary that the organism vary its basal metabolism rate, dilation of the peripheral blood vessels, output of the sweat glands, erection and amount of body hair, and activity of various endocrine glands, as well as other structures and processes. If stability of body temperature is an indication of homeostasis, so then is the lability of the various other factors, for in any homeostatic system, stability at one level is dependent upon lability at others.

If both lability and stability are characteristic of homeostasis, how can one contrast the homeostatic adjustment of two organisms? Which of the multitude of physiological properties of a homeostatic physiology are constant, and which variable? The answer as given by Cannon is quite clear. A homeostatic organism maintains constant those aspects of its physiology whose constancy is necessary for survival. Conjugately, such an organism will be characterized by lability of those processes in which lability is adaptive. These considerations lead to the following definitions of homeostasis as used in a genetic context. A genotype is homeostatic if individuals of this genotype can so adjust their physiology and morphogenesis as to survive and leave offspring in a wide variety of environments. A population or species is homeostatic if its genotypic or phenotypic composition can be so adjusted as to assure its survival in a variety of environments.

Homeostasis, dependent as it is on adaptive value and range of environments for its definition, is a relative term by which genotypes or groups of genotypes may be compared. There is, in this definition at least, no criterion of absolute homeostasis. Moreover, we have deliberately avoided the use of the term "constancy," but refer only to the ability to adjust adaptively to varying environments. This is the essence of homeostasis, at least as we understand it.

A consequence of the foregoing definition is that a direct measure of homeostasis is the mean adaptive value of a genotype over a determined set of environments. Operationally, then, one genotype is defined as being more homeostatic than another if its mean adaptive value is higher in the specified environmental range. In actual practice the choice of environments for a test must be arbitrary and their relation to the multiplicity of environments in nature can only be guessed at.

THE FORMS OF HOMEOSTASIS

Homeostasis may be either collective or individual. An example of collective homeostasis is balanced polymorphism, whereby the continuity of a population through time is assured by the presence in the population of a variety of differently adapted genotypes. Another mechanism of col-

lective homeostasis is found in the rapid reproductive rate of bacteria which allows rapid change in the genetic constitution of the population through mutation and selection. These modes of collective homeostasis are not to be confused with the "genetic homeostasis" described by Lerner (1954). What Lerner discusses is the property of a population to resist sudden changes in its genotypic composition. These changes would presumably be due, for the most part to the occurrence of new selective forces. Such a resistance may often be adaptive in the sense that populations which responded too readily to every short term change in selective force, might easily be trapped in an evolutionary *cul-de-sac*, and then extinguished. This would be the case if all of the genetic variation of the population were lost in a rapid response to a unidirectional selective force. On the other hand, the consequences of resistance to change may just as easily be non-adaptive. Such was the case with some of the populations described by Mather and Harrison (1953). The resistance to selection manifested by these populations resulted in their extinction. If we are to maintain any adaptive criterion of homeostasis, a property which leads to extinction cannot be included under this rubric. Perhaps this concept of Lerner's is better thought of as "genetic impedance" by analogy to that property of electrical circuits which produces both a time lag and a dampening effect on the magnitude of oscillations in an electric current which is subjected to fluctuations in electromotive force. As Lerner points out, his concept is similar to, if not identical with "genetic inertia" of Darlington and Mather (1949). It is also related to the "genetic stability" of Thoday (1953) which is simply the like-produces-like property of genetic systems. It is accomplished by the reproductive stability of the gene, by linkage, by whatever mechanisms exist to prevent the rapid appearance of new genetic variation. This stability, although anti-evolutionary in one sense, is essential to evolution. For, if genotypes do not have some continuity in time, it is impossible for natural selection to act in any directed fashion.

As evolution progresses homeostatic mechanisms tend to change from collective to individual ones. As has been mentioned, bacteria depend in large part on a rapid reproductive rate for their adaptation. This is not to say that individual homeostasis is entirely lacking in bacteria, for indeed, adaptive enzyme formation is an example of individual accommodation. As in all biological generalizations there are many exceptions. Nevertheless the overall trend, if not the chief product of evolution is individual homeostasis. This is simply the effect of stabilizing selection discussed at great length by Schmalhausen (1949).

To achieve individual homeostasis an organism has two usually conflicting forces to deal with. During its morphogenesis it must adapt to whatever forces impinge upon it, yet at the same time it must have the ability to produce an adult morphology and physiology capable of adapting to conditions which may not have been present during its embryonic life. The organism may meet the challenge of fluctuating environment during

morphogenesis by a diversity of morphogenetic pathways, each of which is adaptive in a particular environment. Should such a system of alternatives exist, however, the end product of many of them might be an ill-adapted adult. Waddington's "canalization" (1953) is the process of restriction of the pathways in order to assure the adult of an adaptive morphology. As such, it is a solution to only half the problem for in its effort to do or die, the embryo may die. "Developmental homeostasis" (Lerner, 1954) and "developmental flexibility" (Thoday, 1953) seem to cover both of these processes. Thus Thoday writes:

"An individual may be said to possess developmental flexibility either....if it can develop different phenotypes in different environments, each phenotype better adapted than the others to the environment that evokes it, or if its....development is buffered against environment variables and hence apparently the same adaptive phenotype results in a range of environmental conditions."

The first alternative is that of the adaptation of the embryo, the second, of the adult. This does not mean that a choice between these alternatives exists. An organism, if it is to be homeostatic, must do both. A simple example will illustrate this conflict. If *Drosophila* larvae are grown under starvation conditions, they will produce a fair number of quite small pupae, which in turn eclose as small adults. The production of these smaller than normal flies is an adaptive response to the lack of food. However, these diminutive adults are not fully fertile. Whether it would be of advantage to produce very few, fully fertile adults, rather than a larger number of smaller, semi-sterile individuals can only be answered in a specific case. But clearly adaptation in embryonic and post-embryonic life are opposed in this case.

It is this general problem to which both intra-uterine development in mammals and persistent meristematic tissue in plants, are solutions.

VARIABILITY AS A MEASURE OF HOMEOSTASIS

It might be supposed that since homeostasis involves constancy, at least by implication, that some measure of variability might serve as a criterion of homeostasis. In fact no measure of variability alone is sufficient. That this is so may be seen by considering variation of two sorts, one in physiological and morphological traits, the other in adaptive value itself.

The ambiguity involved in using morphological or physiological variation as an index of homeostasis has already been discussed. Some characteristics of a homeostatic physiology vary while others remain constant. Moreover, this relationship is a causal one. In any system of dynamic equilibrium the constancy of one feature of the system is made possible only by variation in other features. Without some previous information as to the adaptive significance of variation in a given character, the existence of lability or stability *per se* can give no information on homeostasis. Presumably if such previous information did exist, a measure of variability would be pertinent. Ultimately, however, such information can come only

from actual experiment on the adaptive consequences of variation in the character. Ultimately, then, the characterization of a genotype as homeostatic is dependent upon the observation of its adaptive value in a variety of environments.

Both Lerner (1954) and Thoday (1953) point out that only characters of adaptive significance ought to be used in measuring homeostasis. Despite this, both authors make abundant use of evidence on the relative variance in wing length and bristle number in *Drosophila* as evidence that heterozygotes are more homeostatic than homozygotes. Moreover, Lerner, when citing evidence based upon characters of obvious adaptive significance, does not make the important distinction between characters which are components of adaptive value and those whose *variation over a range of environments* is a component of adaptive value. Thus egg weight in poultry is undoubtedly of adaptive significance, but whether it is of advantage to have egg weight vary with environment or remain constant is not clear. Indeed, arguments might be advanced for either alternative.

Turning to variation in adaptive value itself, the situation is somewhat more complex. The relation between mean and variance in adaptive value can best be seen by the following artificial example. Six different genotypes are tested in four environments. Their relative adaptive values in each environment are recorded in table 1 along with the average adaptive value, \bar{W} , and the variance in adaptive value, σ_w^2 , for each genotype. We may assume that the genotypes are equally frequent in each environment, or else each adaptive value may be weighted by the probability that a genotype will be found in a particular environment. This would account for cases in which individuals actually sought out environments to which they were best adapted. Using mean adaptive value as the criterion of homeostasis, the relative homeostasis of the genotypes in table 1 would be

$$A > B > C > D = E > F$$

On the other hand if it were assumed that low variance in adaptive value signified greater homeostasis, the order of the genotypes would be

$$F = D = B > E > C = A$$

which is clearly not consonant with the first ordering. That a comparison based upon constancy alone is unsatisfactory is obvious, for such a system would assign to genotype F a high value of homeostasis. No definition which terms a lethal genotype homeostatic is admissible for it completely traduces the meaning of this concept. An ordering based upon mean adaptive value, however, avoids such difficulties although it leads to a result which at first sight seems unorthodox. Thus, under our definition, genotype A is more homeostatic than genotype B despite the higher variance of the former. In addition genotypes D and E are equally homeostatic although they possess quite different variances in adaptive value. Such a result may be disquieting but if homeostasis is to be any measure of the evolutionary future of a genotype, of the capacity of a genotype to

TABLE 1
POSSIBLE RELATIVE ADAPTIVE VALUES OF 6 GENOTYPES TESTED IN
4 ENVIRONMENTS. \bar{W} IS THE MEAN ADAPTIVE VALUE AND σ_w^2 IS
THE VARIANCE OF ADAPTIVE VALUES.

Genotypes	Environments				\bar{W}	σ_w^2
	I	II	III	IV		
A	7	4	4	2	4.25	4.25
B	4	4	4	4	4	0
C	6	4	4	1	3.75	4.25
D	3	3	3	3	3	0
E	5	3	3	1	3	2.67
F	0	0	0	0	0	0

adapt on the average to varying environment, no other result seems permissible.

Dobzhansky and Wallace (1953) have studied the variance in viability of genotypes homozygous or heterozygous for the gene contents of various chromosomes in several species of *Drosophila*. In their summary they state:

"The homozygotes often show significantly different survival rates in the varying environments of the replicate cultures. Conversely, the heterozygotes gave usually uniform survival despite the environmental variations. It is inferred that the homeostatic adjustments are superior than in homozygotes."

On the basis of this statement it would seem that the criterion of homeostasis which these authors have used is that of variance rather than of mean adaptive value. Nevertheless, what is significant in their data is that heterozygotes show higher mean adaptive values as well as lower variances. Their conclusions about homeostasis are actually based upon this association. If such an association were the rule, no conflict would exist between mean and variance as criteria of homeostasis. In terms of the artificial example described above, genotypes would be either of type B or of type E. That this association is not the rule, is clear from two considerations. First, lethals have both the lowest possible mean adaptive value and the lowest possible variance in adaptive value. Ignoring this objection as being simply a special case, a second objection is apparent when some of the data upon which Dobzhansky and Wallace based their conclusions is examined. Figure 1 is based upon data taken from Dobzhansky and Spassky (1944) on the viabilities of fourth chromosome homozygotes in *Drosophila pseudoobscura*. The viability of these homozygotes was tested at three temperatures. The abscissa in figure 1 represents the mean viability of a given genotype over the three temperatures, while on the ordinate are plotted the values of chi-square as a measure of the varia-

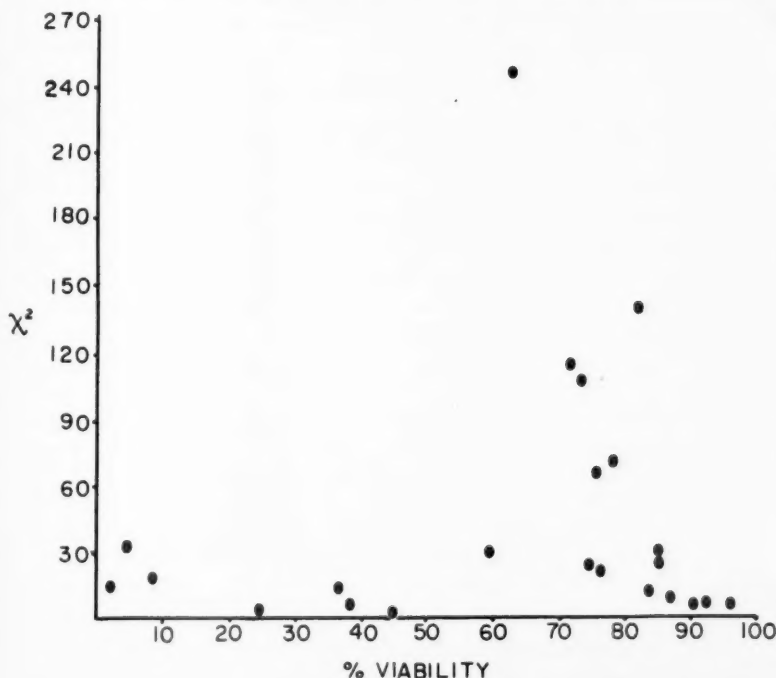


FIGURE 1. Scatter diagram showing the relation of mean viability over three temperatures to the χ^2 between temperatures in fourth chromosome homozygotes of *D. pseudoobscura*. Data taken from Dobzhansky and Spassky (1944).

tion in viability from temperature to temperature. Viability is expressed as per cent of normal; normal being defined as the mean viability of heterozygotes. The points show that small variation is associated both with high and with low mean value. At intermediate viabilities, both high and low values of χ^2 are obtained. The two lowest values of χ^2 are not associated with high viability at all, but with mean values of 45 per cent ($\chi^2 = 0.7$) and 24 per cent ($\chi^2 = 2.6$). This is a graphic demonstration that there is no one to one correspondence between viability and variance.

There is no conflict between this result and the conclusions of Dobzhansky and Wallace. A comparison of figure 1 with figure 2 shows why this is so. This latter curve is based upon data taken from Dobzhansky and Spassky (1954). It shows the frequency distribution of the viabilities of a random sample of homozygotes for the second chromosome of *Drosophila prosaltans*, as compared to that of heterozygotes. The general pattern of this distribution is not confined to this particular chromosome or species, but is repeatedly found whenever a similar test is performed, at least in *Drosophila*. Heterozygotes are confined to that region of the distribution where there is both high mean viability and low variance.

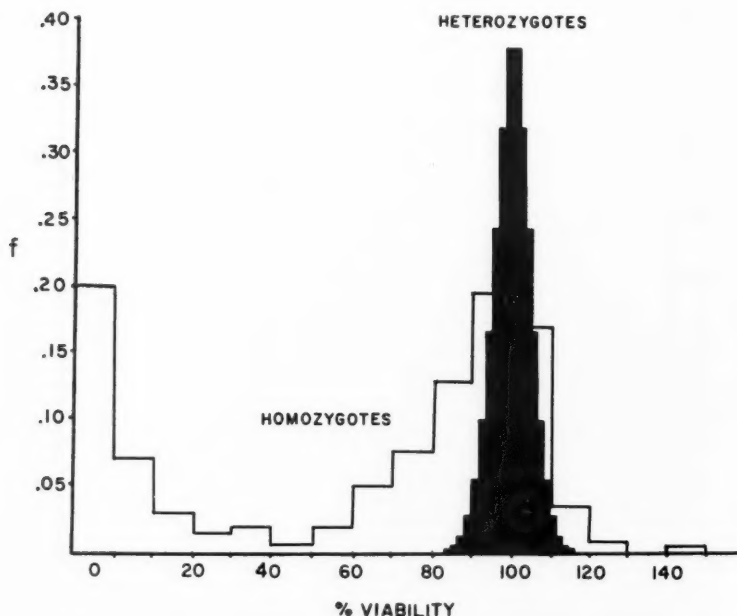


FIGURE 2. Frequency distribution of viability for second chromosomes of *D. prosaltans*. Homozygotes: open bars; heterozygotes: solid bars. Data from Dobzhansky and Spassky (1954).

Homozygotes, on the other hand, have not only a lower mean viability, but are concentrated in regions where both high and low variances exist. Presumably, if heterozygotes could be found which had lower mean viabilities, some of them would show higher variances just as do homozygotes. More important is the fact that homozygotes with high mean viabilities show variances quite as small as do heterozygotes. Small variance in viabilities is not confined to heterozygotes, nor does it give any index of the average fitness of a genotype for it may be observed at any value of viability from lethal to supervital.

The lack of high values of χ^2 in the region between 10 and 50 per cent mean viability makes any theoretical explanation of the observations in figure 1 quite difficult. What is required now is information on a large number of homozygotes covering the entire range of viabilities.

To recapitulate, the lack of one to one correspondence between average fitness and variance of fitness makes it impossible to use this variation as a measure of homeostasis. Rather, a high average fitness itself is a necessary and sufficient criterion of homeostasis. In our view it is the only valid one. In addition, variance in a morphological or physiological character cannot be taken, *per se*, as a measure of homeostasis, but must be coupled with a knowledge of the adaptive meaning of such variation.

It is this problem of the relation between morphological variation and homeostasis which we shall investigate in this and following papers.

MATERIALS AND METHODS

Eighteen strains of *D. melanogaster* homozygous for sub-vital or semi-lethal gene complexes were used in this experiment. In deriving the strains from flies maintained in population cages, each was made homozygous for a different second chromosome by a method described by Wallace (1952). We are indebted to Dr. Wallace and his collaborators for providing samples from their populations. The strains were characterized as to their egg hatchability and larval viability under a variety of population conditions. The results of these tests are described by Lewontin (1955). The numbering of the strains in the present paper is consistent with the previous publication to facilitate comparison.

From the eighteen homozygous strains, eighteen F_1 strains were derived by the following cyclical design. If the homozygous lines are designated A, B, C, R, the crosses are made

$$\begin{array}{l} \sigma\sigma A \times \varphi\varphi B \\ \sigma\sigma B \times \varphi\varphi C \\ : \\ : \\ : \\ \sigma\sigma Q \times \varphi\varphi R \\ \sigma\sigma R \times \varphi\varphi A \end{array}$$

Each homozygous line enters two F_1 crosses, once as a male parent and once as a female. Each F_1 was then mated *inter se* to produce an F_2 generation. Five replicate cultures of each homozygote and each cross were obtained by serial transfer of a single group of parents every 48 hours. After 48 hours in the last bottle the parents were discarded. No particular attempt was made to standardize replicate bottles as some environmental variation was desired. All cultures were kept at $20^\circ \pm 0.5^\circ\text{C}$.

From each replicate ten males and ten females were examined and the number of bristles on the fourth and fifth sternites were counted. No individual placed in position for counting was rejected except a few cases where tumors or an abnormal abdomen distorted the bristle bearing area. In this way unconscious selection on the basis of bristle number was avoided.

It should be pointed out that the homozygote, F_1 and F_2 series were made from three to four weeks apart and thus were not strictly simultaneous. Within each set, however, all strains were transferred simultaneously for replications. These replications covered a period of ten days and actual examination covered the full three to four weeks between sets. Thus, it is not likely that temporal changes in experimental conditions from the beginning to the end of the experiment contributed to the differ-

ences observed between homozygotes, F_1 and F_2 . It will be seen that the consistency of results bears out this point.

ESTIMATES OF VARIANCE

What is being sought in this experiment is the effect of varying environments on the phenotype of the strains investigated. A direct measure of this effect is the variance in bristle number apportionable to environmental differences. There are several ways in which this environmentally determined variance can be estimated. First, the variance between individuals within replicate cultures may be used. Designating this variance as σ_w^2 , it will be compounded of the following elements:

$$(1) \quad \sigma_w^2 = \sigma_G^2 + \sigma_e^2 + \sigma_{Ge}^2 + \sigma_t^2 + \sigma_{te}^2$$

where σ_G^2 = genetic variance due to segregation on the second chromosome

σ_e^2 = variance due to micro-environmental variation

σ_t^2 = genetic variance due to segregation of the residual genotype

and σ_{Ge}^2 and σ_{te}^2 are the interaction variances between the appropriate factors. Now for homozygotes and F_1 crosses σ_G^2 will be zero. Moreover, the method of extracting the second chromosome is such that the residual genotypes are on the average the same for all strains and between crosses of the same strain. This being so, one can ignore the last two terms in expression (1) when comparing variances of different strains or crosses. The only two terms left are the micro-environmental variance and the interaction variance between genotype and micro-environment, these being the components of interest.

A second estimate may be derived from the variance between means of the five replicates in each cross. Designating this variance as σ_b^2 we see that

$$(2) \quad \sigma_b^2 = \sigma_E^2 + \sigma_{GE}^2 + \frac{1}{n} \sigma_w^2$$

where σ_E^2 = variance due to differences in environment between replicates and n = number of individuals measured in each bottle.

Since n is known and we have already estimated σ_w^2 , we have only to substitute them in (2) to get an estimate of the effects of bottle-to-bottle variation in environment and its interaction with the genotype.

A third variance estimate of interest is of a somewhat different nature. For any individual measured, segments four and five in general have different numbers of bristles, segment five on the average more than segment four. In any individual this gradient may be reversed. Using the replication means as a measure of the average difference between the two segments, the variance of this difference from individual to individual is calculated. This variance is approximately related to the correlation $r_{4,5}$ between these segments by the expression

$$(3) \quad \sigma_d^2 \approx (\sigma_4^2 + \sigma_5^2) (1 - r_{4,5}) \quad \text{when} \quad \sigma_4^2 \approx \sigma_5^2$$

We have chosen to use σ_d^2 rather than $r_{4,5}$ because the former increases with increasing effect of environment as do the other two variance estimates, while the correlation changes in the opposite sense. In addition $r_{4,5}$ depends upon the variance in total bristle number while σ_d^2 is free of the effects of this variance. Thus, σ_d^2 can be compared with less confusion. It is simply a measure of the lack of correlation between the two segments of an individual.

RESULTS

Tables 2, 3, and 4 show the mean number of bristles and the variance components just described for homozygous, F_1 and F_2 crosses. Males and females are tabulated separately since they have quite different average bristle numbers. The values of σ_w^2 and σ_d^2 represent pooled estimates

TABLE 2
MEAN ABDOMINAL BRISTLE NUMBER AND VARIANCE COMPONENTS FOR
HOMOZYGOTES, F_1 AND F_2 IN MALES. SEE TEXT FOR
EXPLANATION OF SYMBOLS.

Strain	\bar{X}		σ_w^2		σ_b^2		$\sigma_E^2 + \sigma_{GE}^2$	
	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2
I	36.28		10.09		2.65		1.64	
I x II	35.26	36.78	12.80	7.98	3.25	1.85	1.97	1.05
II	35.58		5.58		.71		.16	
II x 18	34.34	34.58	11.31	8.18	2.36	.71	1.23	-.11
18	34.44		4.29		.41		-.02	
18 x 22	31.56	33.62	9.89	5.58	3.92	.38	2.93	-.18
22	30.10		7.39		2.14		1.40	
22 x 25	31.96	30.60	7.49	6.47	2.93	.83	2.18	.18
25	32.36		6.07		1.69		1.08	
25 x 27	33.86	31.28	7.18	5.47	1.87	4.44	1.16	3.90
27	32.18		5.47		1.34		.80	
27 x 28	35.22	33.74	10.53	12.93	1.23	1.24	.17	-.06
28	35.10		10.98		1.06		-.04	
28 x 33	36.26	34.08	10.49	5.82	1.49	.75	.44	.17
33	34.76		6.73		.84		.17	
33 x 34	33.84	34.90	5.80	10.00	1.98	1.45	1.40	.45
34	32.84		6.73		1.38		.71	
34 x 35	34.06	33.66	7.40	6.31	.40	.64	-.34	.01
35	35.06		5.47		.99		.45	
35 x 41	34.14	33.52	8.09	15.42	1.80	2.70	.99	1.16
41	31.84		5.96		1.89		1.30	
41 x 45	32.74	34.30	9.13	5.36	.32	3.56	-.59	3.03
45	34.48		5.29		.34		-.19	
45 x 47	33.04	33.92	4.56	4.53	3.42	3.48	2.97	3.03
47	32.26		5.04		.32		-.18	
47 x 48	34.98	32.80	8.78	9.04	2.64	.69	1.77	-.22
48	32.40		8.80		4.85		3.97	
48 x 54	35.22	32.48	9.87	8.80	.42	.65	.57	-.23
54	32.20		8.32		.44		-.40	
54 x 62	35.06	34.32	6.07	7.33	.64	1.02	.03	.29
62	34.96		6.91		1.12		.43	
62 x 67	33.72	32.42	5.22	9.04	3.38	1.19	2.86	.28
67	33.00		8.32		2.59		1.75	
67 x 74	34.94	34.42	6.31	9.24	.52	.84	.11	-.08
74	35.90		7.88		.71		-.08	
74 x I	34.36	34.60	8.18	6.36	1.99	4.90	1.17	4.27
I	36.28		10.09		2.65		1.64	

TABLE 3

MEAN NUMBER OF ABDOMINAL BRISTLES AND VARIANCE COMPONENTS FOR HOMOZYGOTES, F_1 AND F_2 IN FEMALES. SEE TEXT FOR EXPLANATION OF SYMBOLS.

Strain	\bar{X}		σ_w^2		σ_b^2		$\sigma_e^2 + \sigma_{GE}^2$	
	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2
I	45.02		10.76		5.15		4.07	
I x II	43.62	45.98	17.18	20.22	9.53	3.63	7.81	1.61
II	43.28		10.80		2.01		.93	
II x 18	41.92	43.28	11.29	12.58	4.94	.25	3.81	-1.01
18	42.06		6.87		.57		-.11	
18 x 22	39.52	42.26	1.49	12.62	.51	1.34	.36	.08
22	38.02		5.04		2.70		2.20	
22 x 25	38.82	37.54	11.04	7.20	2.97	.92	1.86	.20
25	37.06		9.11		.83		-.08	
25 x 27	39.80	38.52	9.36	9.24	.77	.35	-.17	-.57
27	40.80		8.20		.45		-.37	
27 x 28	48.86	42.20	9.53	16.87	2.82	3.47	1.87	1.78
28	43.20		9.31		3.42		2.48	
28 x 33	45.20	41.96	14.44	14.98	.42	2.20	-1.03	.70
33	42.54		8.40		3.35		2.51	
33 x 34	41.30	40.92	17.44	14.36	.74	1.90	-1.00	.46
34	39.06		10.64		3.84		2.77	
34 x 35	38.89	40.22	9.47	7.09	.73	.94	-.22	.23
35	40.74		10.40		2.28		1.24	
35 x 41	40.20	40.04	8.67	13.89	4.25	.82	3.38	.57
41	39.24		8.00		1.26		.46	
41 x 45	41.18	44.14	6.63	13.64	1.04	2.45	.40	1.09
45	43.22		6.87		3.69		3.00	
45 x 47	40.80	40.76	5.78	13.64	3.00	7.83	2.42	6.46
47	39.16		4.71		.52		.05	
47 x 48	44.08	42.32	9.93	22.07	2.26	2.85	1.27	.65
48	39.92		9.29		3.41		2.48	
48 x 54	43.40	41.30	16.96	13.33	1.08	3.11	-.62	1.77
54	39.46		10.80		1.63		.55	
54 x 62	41.26	42.04	15.84	20.64	5.02	3.03	3.44	.96
62	42.90		13.64		2.89		1.53	
62 x 67	41.04	39.02	10.82	11.49	4.83	1.11	3.75	-.04
67	39.34		8.00		5.08		4.28	
67 x 74	41.34	42.96	20.49	14.20	1.39	2.02	-.66	.60
74	44.44		11.34		1.21		.08	
74 x I	44.04	43.40	17.62	11.16	6.97	3.11	5.21	1.99
I	45.02		10.76		5.15		4.07	

from the five replications. Table 5 shows a condensed comparison among the homozygous, F_1 and F_2 crosses, the entries in which are simply the grand totals of the appropriate columns in tables 2, 3, and 4.

The first thing which is clear from table 5 is that there is little or no heterosis in the sense used in quantitative genetics ("luxuriance" in the terminology of Dobzhansky, 1952). The F_1 crosses show a very slight, nonsignificant increase in mean bristle number over the homozygotes. The F_2 values drop back again, in the case of males to the parental value, in the case of females to an intermediate one. The genes seem to be acting in an additive fashion with perhaps a small amount of dominance.

Inspection of the variances shows a more interesting picture. In both sexes σ_w^2 and $\sigma_E^2 + \sigma_{GE}^2$ are higher in F_1 than in homozygotes. On the other hand σ_d^2 is lower for heterozygotes. Table 6 shows the value of the

variance ratios (F) for the various comparisons along with the appropriate degrees of freedom and probability levels. The F values shown for comparing the values of $\sigma_E^2 + \sigma_{GE}^2$ have been calculated in a special way. Because they are derived by subtracting one estimate of variance from another they cannot be tested by the usual F ratio. Instead we have used a method suggested by Cochran (1951) to which Mr. Gilbert Paul has drawn our attention. The ratio

TABLE 4
VARIANCE OF THE DIFFERENCES BETWEEN SEGMENTS 4 AND 5 (σ_d^2)
IN HOMOZYGOTES, F_1 AND F_2 MALES AND FEMALES.

Strain	Males			Females		
	Hom.	F_1	F_2	Hom.	F_1	F_2
1	4.76			6.93		
		3.73	4.16		4.29	5.11
11	4.91			4.89		
		4.16	6.44		4.89	6.89
18	4.16			5.44		
		4.24	3.89		4.31	3.56
22	2.25			3.93		
		5.53	5.18		5.27	5.11
25	4.02			6.36		
		2.87	4.53		5.27	4.49
27	3.87			5.13		
		5.56	4.09		4.69	6.51
28	6.69			5.71		
		3.64	4.84		4.78	4.67
33	5.22			5.78		
		4.51	3.02		6.11	2.93
34	5.58			7.18		
		3.67	3.64		3.47	5.84
35	6.80			5.56		
		4.31	5.29		4.00	4.51
41	3.82			7.38		
		4.11	2.47		4.62	4.40
45	3.29			5.67		
		5.62	4.13		5.38	4.36
47	3.76			6.62		
		3.67	3.13		5.67	5.89
48	3.29			9.47		
		3.73	3.64		5.89	5.33
54	4.82			12.07		
		2.96	6.18		8.96	6.64
62	3.44			6.13		
		3.00	3.62		3.93	4.20
67	4.09			4.22		
		5.51	2.67		4.18	4.60
74	6.53			8.39		
		2.18	6.62		4.47	6.49
1	4.76			6.93		

TABLE 5
CONDENSED COMPARISONS OF HOMOZYGOTES, F_1 AND F_2 . ENTRIES
ARE TOTALS FROM TABLES 2, 3, AND 4.

		\bar{X}	σ_w^2	σ_b^2	$\sigma_E^2 + \sigma_{GE}^2$	σ_d^2
Males	P	606.10	125.32	25.47	12.93	81.30
	F_1	614.56	140.92	34.56	19.67	72.98
	F_2	606.02	143.87	31.32	16.93	77.55
Females	P	739.06	162.18	44.30	28.07	116.86
	F_1	756.27	213.98	53.25	31.88	90.16
	F_2	748.86	249.21	41.31	16.38	91.53

$$(4) \quad F' = \frac{\sigma_{b_1}^2 + \frac{1}{10} \sigma_{w_2}^2}{\sigma_{b_2}^2 + \frac{1}{10} \sigma_{w_1}^2}$$

is used. This has degrees of freedom

$$(5) \quad \frac{\left[\sigma_{b_1}^2 + \frac{1}{10} \sigma_{w_2}^2 \right]^2}{\frac{(\sigma_{b_1}^2)^2}{N_1} + \frac{\left(\frac{1}{10} \sigma_{w_2}^2 \right)^2}{N_2}} \text{ and } \frac{\left[\sigma_{b_2}^2 + \frac{1}{10} \sigma_{w_1}^2 \right]^2}{\frac{(\sigma_{b_2}^2)^2}{N_1} + \frac{\left(\frac{1}{10} \sigma_{w_1}^2 \right)^2}{N_2}}$$

in the numerator and denominator, respectively. N_1 and N_2 are the number of degrees of freedom for σ_b^2 and σ_w^2 .

As table 6 shows, σ_w^2 is significantly higher in heterozygotes than in homozygotes. σ_b^2 and $\sigma_E^2 + \sigma_{GE}^2$ are also higher but not significantly so. This is presumably due to the much smaller number of degrees of freedom available for these comparisons, with a resulting decrease in sensitivity of the test. σ_d^2 on the other hand, is significantly lower in F_1 than in the parents. In the F_2 , the within-bottle variances are even higher than in F_1 . This is to be expected when it is remembered that the F_2 is the first generation in which genes on the second chromosome are segregating. Despite this increase, however, the values of σ_b^2 are lower than in the F_1 . The result is a marked, consistent, but not significant decrease in the estimate of $\sigma_E^2 + \sigma_{GE}^2$. Finally the F_2 values of σ_d^2 are intermediate in value between homozygotes and F_1 .

There is one other item of information which is pertinent to this study. As has been mentioned there is an average anterior-posterior gradient in bristle number, the posterior segment generally having more bristles than the anterior. Table 7 shows the mean difference between these segments expressed as (posterior-anterior). For heterozygotes, all of these differ-

TABLE 6
F-RATIOS, DEGREES OF FREEDOM AND PROBABILITY LEVELS FOR
COMPARISONS OF HOMOZYGOTES, F_1 AND F_2
VARIANCES IN TABLE 4.

				Males			
				σ_w^2	σ_b^2	$\sigma_e^2 + \sigma_{ce}^2$	σ_d^2
P - F ₁	F			1.12	1.36	1.19	1.11
	d. of freedom	num.		810	72	132	810
		den.		810	72	169	810
	Pr.			.05	.10	.10-.20	.05-.10
F ₁ - F ₂	F			1.02	1.10	1.08	1.06
	d. of freedom	num.		810	72	142	810
		den.		810	72	149	810
	Pr.			>.20	>.20	>.20	.20
P - F ₂	F			1.15	1.23	1.10	1.05
	d. of freedom	num.		810	72	139	810
		den.		810	72	171	810
	Pr.			.01-.05	.10-.20	>.20	>.20
Females							
				σ_w^2	σ_b^2	$\sigma_e^2 + \sigma_{ce}^2$	σ_d^2
P - F ₁	F			1.32	1.20	1.06	1.30
	d. of freedom	num.		810	72	121	810
		den.		810	72	155	810
	Pr.			<.001	.10-.20	>.20	<.001
F ₁ - F ₂	F			1.17	1.29	1.25	1.02
	d. of freedom	num.		810	72	152	810
		den.		810	72	161	810
	Pr.			.01-.05	.10-.20	.05-.10	>.20
P - F ₂	F			1.54	1.07	1.20	1.28
	d. of freedom	num.		810	72	171	810
		den.		810	72	137	810
	Pr.			<<.001	>.20	.10	<.001

TABLE 7
MEAN DIFFERENCE IN ABDOMINAL BRISTLE NUMBER BETWEEN
SEGMENTS 4 AND 5 EXPRESSED AS (5-4) IN HOMOZYGOTES,
F₁ AND F₂ CROSSES.

Strain	Males			Females		
	Hom.	F ₁	F ₂	Hom.	F ₁	F ₂
I	3.4			1.5		
I x II		4.3	4.9		4.3	2.3
II	2.5			3.5		
II x 18		6.1	3.7		3.8	4.0
18	5.6			3.2		
18 x 22		5.4	4.9		5.2	.3
22	3.4			3.5		
22 x 25		8.0	3.2		4.5	4.3
25	6.2			2.1		
25 x 27		5.5	3.4		1.8	2.4
27	1.7			-.6		
27 x 28		2.3	1.1		3.3	2.8
28	.6			2.2		
28 x 33		6.3	3.2		5.6	5.2
33	1.2			3.9		
33 x 34		4.8	4.7		3.9	2.8
34	3.6			7.7		
34 x 35		3.9	3.9		1.6	2.5
35	.3			.5		
35 x 41		4.1	4.8		4.4	3.4
41	2.2			6.3		
41 x 45		4.1	3.3		4.9	1.3
45	5.0			2.9		
45 x 47		5.0	1.6		4.8	.8
47	3.5			1.4		
47 x 48		3.9	1.6		3.8	.6
48	-1.6			-.6		
48 x 54		4.1	2.4		5.6	1.9
54	-.7			3.0		
54 x 62		3.3	4.6		2.9	.2
62	4.6			-.1		
62 x 67		5.4	4.9		1.2	2.9
67	1.8			3.6		
67 x 74		8.5	3.3		3.3	2.6
74	3.4			5.3		
74 x I		7.8	3.6		5.4	-1.2
I	3.4			1.5		
σ^2	3.93	2.96	1.35	5.11	1.96	2.39

ences are positive and fairly uniform. For homozygotes, however, some are negative and they are considerably more variable. If the values for the variances are pooled for males and females, homozygotes have a significantly higher variance than heterozygotes ($P = .05$). If the individual values for replicate cultures are examined the same phenomenon appears.

Among 180 homozygous cultures there are 38 negative differences, while among a like number of heterozygous cultures there are only 11, the F_2 being intermediate with 31 negative values. Homogeneity tests on the number of negative and positive values for the three types of crosses show the F_1 to be significantly different from the homozygotes and F_2 ($P < .001$ in both cases) while the F_2 does not differ significantly from the homozygotes ($P = .25$).

In summary, heterozygotes show a higher variance between individuals than do homozygotes, but a lower variance, or higher correlation, between segments. The F_2 is generally intermediate between the homozygotes and F_1 except for the variance within bottles which contains genetic variance due to segregation on the second chromosome.

DISCUSSION

As has been previously stated, the purpose of this investigation has been to examine the relationship between homeostasis and morphological variation. The homeostatic properties of the various genotypes used in these experiments have already been established, both in the initial isolation of genotypes and in a more detailed analysis of their viability and egg-hatchability values by Lewontin (1955). The various homozygous strains are emphatically lacking in homeostatic properties when compared with heterozygotes. Reference to the experiments just cited shows clearly that for the two important components of adaptive value measured, homozygotes are inferior under a wide variety of population densities. By now it is well established, for *Drosophila* at least, that chromosomal homozygotes are with few exceptions lethal, semi-lethal, or sub-vital as compared with heterozygotes. (Dobzhansky, Holz and Spassky, 1942; Dobzhansky and Spassky, 1943; Wallace, 1952; and Wallace and Madden, 1953).

Accepting then, the greater homeostasis of heterozygotes, we may ask what the morphological correlates of this condition are. The experiments described here show that there is no simple relation between homeostasis and morphogenetic uniformity. If variance in total bristle number is used as a criterion of morphogenetic uniformity, heterozygotes must be considered as more sensitive to environmental variation. Both σ^2_w and $\sigma^2_E + \sigma^2_{GE}$ which are measures of environmentally caused variation are higher in the F_1 than in the homozygotes. On the other hand if the phenotypic correlation between parts of an organism is considered, we find it to be greater in the homeostatic heterozygotes than in the non-homeostatic homozygotes. In our experiments the anterior-posterior gradient is considerably more constant for heterozygous genotypes than for their homozygous parents. Thoday (1953) in his very interesting analysis suggests that just such a relation ought to exist. His main argument is that organisms may adapt to varying environments by varying their phenotype adaptively, but that they must, in the process, maintain a certain harmonious balance between their component parts. This is a tempting concept, but it loses force in the transfer from the experiments he cites, to those with

which we are dealing. Thoday was mainly concerned with bilateral symmetry. It might be argued that it is difficult to imagine many environments in which an asymmetrical *Drosophila* is at an advantage when compared with his symmetrical brothers. However, in many organisms it is precisely the asymmetrical condition which has been established by natural selection. When one deals with an anterior-posterior gradient as we are doing, it is even more doubtful that homeostasis will be accompanied always by a consistent gradient. If variation in total bristle number is an adaptive response why not variation in gradient as well? Much of evolution is concerned with shape changes, such changes being due simply to alterations in morphogenetic gradients.

Our result seems to be in conflict with that of Reeve and Robertson (1954) who studied variance of bristle number in inbred lines and crosses between lines. Their conclusion was that the correlation between segments and the total environmental variance did not differ from inbred lines to heterozygotes. They make a point of the fact, however, that their experiment was performed under "optimal" conditions, which may be the key to the conflict between the results. As they show, under "optimal" (non-crowded) conditions there is very little correlation between the segments, holding genotype constant. When population density was made variable, however, there was a significant correlation between segments. Unfortunately, this part of the experiment was performed only on the heterozygotes, so that no comparison with their inbred lines is possible. It is of course entirely reasonable that under more or less uniform optimal conditions any differences in morphogenic sensitivity to environment will be minimized. The question of morphogenetic stability and homeostasis arises only when there is a multiplicity of environments.

Despite these reservations our experiments do support the following general mode of adaptation. There is phenotypic variability with the maintenance of harmonious proportion between parts of the same organism. For other characters or other organisms the relation may be quite different.

SUMMARY

Homeostasis has been defined in this paper as the property of a genotype or collection of genotypes which allows it to respond adaptively to a wide variety of environments. The measure of homeostasis is then the average adaptive value over a range of environments.

The variance in abdominal bristle number of a series of strains of *Drosophila melanogaster* homozygous for semi-lethal and sub-vital gene complexes has been examined. It is shown that these homozygotes which are less homeostatic than heterozygotes constituted from them, show a lower variance in total bristle number than do the heterozygotes or F_2 populations. On the other hand both the heterozygotes and F_2 populations show a higher correlation in bristle number between the two segments examined. It is suggested in conclusion that no one-to-one correspondence exists between phenotypic variance and homeostasis, although a higher pheno-

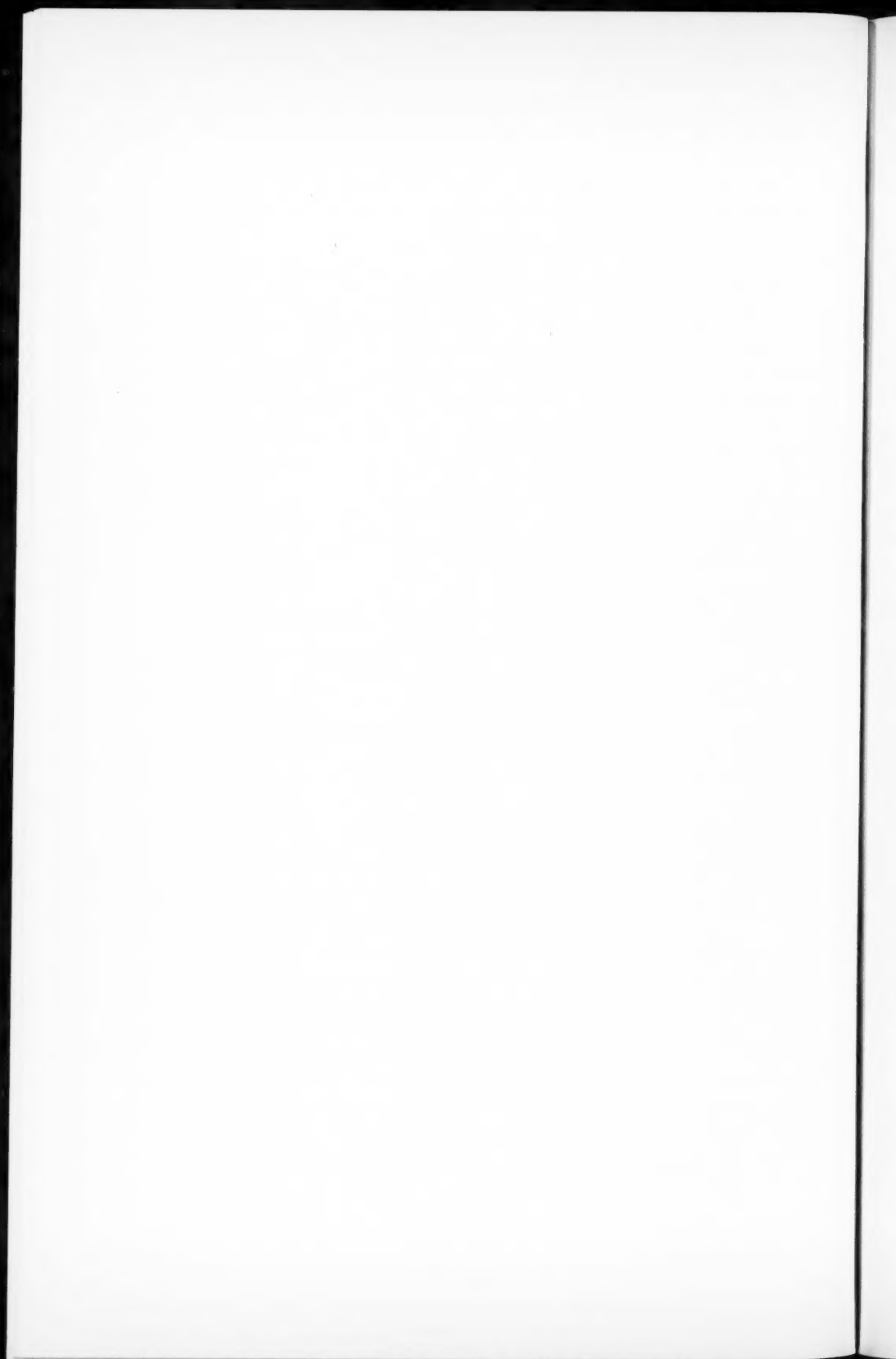
typic correlation between two parts of the same organism might be more often than not a concomitant of superior homeostatic adjustment.

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RECIPROCAL DEPENDENCE OF ECTODERM AND MESODERM
DURING CHICK EMBRYO LIMB DEVELOPMENTEDGAR ZWILLING¹

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INTRODUCTION

The limbs of vertebrates start their development as simple structures consisting of two components: a mesodermal thickening of the somatopleure and a covering of ectoderm. For a considerable period our best information about the relative parts played by these components has come from experiments with amphibian embryos. The investigations of R. G. Harrison and his students have revealed, in the words of Nicholas (1955), that, "The mass of evidence points to the determination of the limb mesoderm of urodeles as the positive factor of limb formation." The experiments on which such a statement are based are those in which limbs have developed after the mesoderm (either in situ or when grafted to a foreign site) has been covered by non-limb ectoderm. When such experiments (Harrison, 1931) were done on *Amblydyoms punctatum* embryos it was found that, with the exception of head ectoderm from neurula and post-neurula stages, all ectoderm from embryos as old as tail bud stages could support normal limb development. These limbs conformed in all respects to the "determiners" of the mesoderm. Only such minor features as digit shape could be ascribed to an ectodermal influence when there was an exchange of components between species (Rotmann, 1933).

Not all of the evidence, even among the amphibia, is consistent with these ideas. Balinsky (1935) found that there was a limited distal development when Triton limb buds were grown free of all ectoderm. The extent of the distal development depended on the stage when the ectoderm was eliminated. Additional positive involvement of ectoderm in limb development was indicated by the work of Steiner (1928) with anura and Filatow (1928) and Balinsky (1931) with urodeles. These experiments are not cited more fully since they certainly lack the conclusiveness of those of the Harrison group.

COMPARATIVE DEVELOPMENT OF LIMB BUDS

Limb buds of amphibia are not structurally the same as those of other vertebrates. In most other vertebrates (mammals, birds, reptiles and even some fish) the early limb buds characteristically have a thickened ridge or crest of ectoderm along the free edge. This has been called the apical ectodermal ridge (Saunders, 1948). Most amphibia have no such structure

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at the free edge. At best there is a thicker cap of ectoderm, similar to the initial stages of a regeneration blastema, at the distal end of the limb. This ectodermal cap may have some importance for continued limb development, at least in anura, but this is frequently not evident because the cap can be regenerated fairly readily following its elimination (Steiner, 1928). The apical ridge of the chick limb bud is not replaced by regeneration (Saunders, 1948). These differences are basic and probably are responsible for a good many of the divergencies of limb development seen when the amphibia and birds are compared.

The importance of the apical ectodermal ridge of the chick embryo's limb bud was shown by Saunders (1948). When the ridge was removed distal development of the limb ceased even though the mesoderm was not injured and the rest of the limb ectoderm quickly healed over the exposed area. The extent of distal development depended on the stage of the limb bud at the time of the operation. Even the ectoderm which was closely adjacent to the ridge could not form a new one. These observations were confirmed in a "wingless" mutant of chickens (Zwilling, 1949). The "wingless" condition was due to a recessive mutation which, in homozygotes, eliminated Saunders' ridge from the wingbuds at an early stage. Distal development of the wings failed, just as in Saunders' experiments.

There have been reasons to believe that Saunders' ridge was somehow involved in the development of other mutant conditions. Bretscher (1950) suspected this as a result of his experiments with polydactylous chickens. T. C. Carter (1954) went to the extent of calling the apical ridge "the limb inductor" when he elaborated an hypothesis to explain the polydactyly in luxate mouse mutants. It was obvious that a complete account of the relative importance of the two limb components, both for mutant conditions and in normal development, could best be obtained by some procedure which would allow for the interchange of ectoderm and mesoderm between limb buds. Such a procedure has been devised (Zwilling, 1955). The present communication is a synthesis of the results obtained from the application of this technique to the development of two mutant conditions as well as to several aspects of the development of genetically normal limbs. The information which has been obtained from the hereditary deviants has a central position in the elaboration of our hypothesis for limb development.

DEVELOPMENT OF LIMBS IN CHICK EMBRYOS

Normal chick embryo limb development has been described by Saunders (1948) and Hamburger and Hamilton (1951). In the earliest stages both leg and wing buds are fairly symmetrical. The ectoderm of the apical ridge at first is more columnar than adjacent ectoderm. Then it becomes similar to a pseudostratified layer of columnar epithelium and forms the typical nipple-like ridge or crest to which we have referred. At stage 17 the more columnar ectoderm of the developing ridge is fairly uniform in thickness along its entire length. This changes quite rapidly and by stages 18 and

19 the ridge is considerably higher (4-5 layers of nuclei) at the apex of the bud than at the anterior and posterior ends where it merges with the body ectoderm. By stage 20 this central thicker or higher region of the ectodermal ridge is very obvious. In addition the leg bud has become asymmetrical: the anterior portion tapers gradually and the posterior part of the bud is more solid and is cut off more abruptly. This foreshadows a posterior bulging of the developing limb. The apical ridge of ectoderm is highest in the region of the bulge and tapers down at both ends—more rapidly at the posterior and gradually at the anterior end. At stage 26 the wing and leg asymmetries are pronounced (Fig. 1). There is a distinct difference between the leg and wing (the former with a more pronounced posterior bulge in the paddle region, the latter with a more extensive anterior bulge) and a difference between the pre- and post-axial edges in each. Again we note that the thicker apical ectoderm is restricted to the

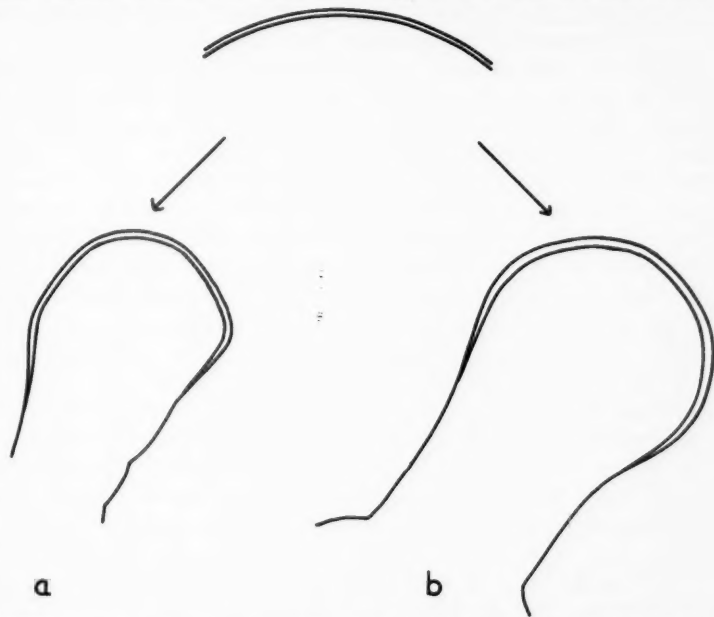


FIGURE 1. Diagram of generalized limb bud at early stage when the apical ectodermal ridge is uniform. This leads to the asymmetries seen in the stage 26 wing (a) and leg (b).

region of new outgrowth (the paddle or presumptive digit region) and corresponds with the limb asymmetries. At this stage we note that the ectoderm along both the pre- and post-axial edges of the 'arm' and 'leg' regions can no longer be distinguished from the thin lateral ectoderm. Special emphasis has been deliberately placed on the asymmetries of the limbs and the associated asymmetries of Saunders' ridge during this description. The reasons for this will be made clear subsequently.

EXPERIMENTAL INTERCHANGE OF PARTS OF LIMBS

Experiments with the interchange procedure (mostly with stage 19 limb buds) have confirmed the importance of Saunders' ridge for limb development (Zwilling, 1955). Distal limb components do not form when the ridge is removed. Ectoderm from various other regions of the body has been placed over the limb mesoderm. The same has been done with ectoderm from younger embryos (5 to 16 somites). In a number of instances the latter ectoderm included the presumptive limb areas. Not a single case of development of distal structures has been obtained. In most cases only atypical girdle elements were found. Occasionally part of a femur or humerus formed. Lateral limb bud ectoderm (not including the ridge) seems to promote distal development somewhat more than ectoderm from other sites (unpublished). When such ectoderm was placed over the apex of a limb bud's mesoderm a fully formed femur or humerus developed. This conforms to Saunders' findings after he removed only the apical ridge. Again there were no cases in which more distal structures formed.

On the positive side we have found that distal limb elements developed whenever the apical ectodermal ridge was successfully fused to limb bud mesoderm. This was the case when the grafted ectoderm merely replaced the original in as nearly the same position as possible. It was also the situation when the apical ridge contacted other regions of the limb bud mesoderm. When the original apical ridge and about .1 to .15 mm of the underlying mesoderm were removed the remainder of the mesoderm could form an entire, fairly normal limb if it were provided with a new apical ectoderm from another limb bud. The strip which consisted of the original Saunders' ridge and underlying mesoderm also developed into a complete limb. Thus two limbs can form from one limb bud if an additional apical ridge is introduced into the system in an appropriate way. Various degrees of duplication of distal limb structures may be obtained if an ectodermal ridge (or part of one) is placed on each lateral surface of the intact mesoderm (deprived of all of its ectoderm) from one limb bud. We have had limbs, from such operations, in which there were only a couple of accessory digits. In others all structures distal to the humerus were duplicated (Zwilling, '56a). Thus, not only is the apical ectodermal ridge required for distal development of a limb, but it can promote or induce outgrowth and distal development from various parts of the limb bud.

EXPERIMENTS WITH LIMB BUDS FROM MUTANTS

Data which we have obtained from experiments with limb mutants indicate that the ectodermal ridge is dependent on the mesoderm for its continued persistence and activity. Mesoderm from a mutant limb was covered by genetically normal ectoderm. The reciprocal combination was also made and the limb buds from both combinations were raised as flank grafts on genetically normal hosts. When the mesoderm from genetically polydactylous buds was covered with normal ectoderm the polydactylous condition developed in the grafts (Zwilling and Hansborough, 1956). This was

found to be true for limbs from two polydactylous mutations: wing buds from duplicate (Po^d) and leg buds from Silkie (Po) embryos. The pattern of the duplication in the grafts was almost identical with that of the control wing. Supplementary observations established that stage 19 duplicate wing buds did not differ in size or appearance from either genetically normal wing buds or phenotypic normals from the duplicate mating. A series of experiments were performed in which a piece of tantalum foil was inserted in a transverse incision through the center of the limb bud. This separated the limb buds into pre- and post-axial halves. When this was done to a normal wing bud only digit #1 developed from the pre-axial half. In the case of duplicate wing-buds digit #1 plus the accessory limb structures developed from the pre-axial half. At the time of the operation the pre-axial halves were identical in appearance in duplicate and normal wing buds. As the pre-axial half of the duplicate limbs developed, it was evident that the thicker more active region of the apical ridge was more extensive pre-axially than in controls. This anterior extension of the ridge was associated with the excessive pre-axial outgrowth and is noted consistently in the developing duplicate limbs.

That accessory limb outgrowth is dependent on the ectodermal ridge is emphasized by experiments in which only the pre-axial region of the ridge was removed from duplicate wing buds. If just the right amount of Saunders' ridge was cut off (Hansborough, unpublished) perfectly normal wings, minus any pre-axial duplication, developed. When all of this evidence is evaluated one has the following sequence of events: 1. at first neither the ectoderm nor mesoderm of polydactylous limb buds differ from the normal. 2. polydactylous limbs develop a greater pre-axial outgrowth than normal. This has associated with it a more extensive active apical ectodermal ridge which induces the excess outgrowth. 3. The same sort of extension of the apical ridge is developed in genetically normal ectoderm when it is in contact with polydactylous mesoderm and the typical accessory digits form. There is only one conclusion from these facts, namely that the extension of the thicker apical ectoderm of the ridge results from some influence present in the mutant mesoderm and absent in genetically normal limb mesoderm.

Similar combinations of mutant and genetically normal limb bud components have been made with wing buds from embryos homozygous for a wingless mutation (Zwilling, '56a). Wing buds appear at the usual time in such homozygotes. An apical ectoderm which is typical for stage 17 or 18 is present. But it never progresses beyond this stage, never becomes thicker. Instead it regresses and is completely absent by stage 23 or 24 (by the fourth day). Typically the only trace of the wing is the girdle—which develops reasonably well. Some of the homozygotes also have abnormal legs. However the apical ectodermal ridge persists for a longer time in the leg buds so that more of the leg develops. Frequently there is a normal thigh and the limb ends abruptly somewhere along the tibia, which is spike-like. Occasionally there are deficient digits. The cessation of

distal development is linked to the time when the apical ridge regresses. When mesoderm from homozygous "wingless" wing buds was covered by genetically normal ectoderm and grafted to genetically normal hosts the results were quite consistent. In each case the wing developed more than did the control (the unoperated wing left in situ in the donor). But in each case the genetically normal apical ridge regressed sooner or later and distal development stopped. The limbs were spike-like structures with an abruptly terminated ulna. In one or two cases there was an attempt at the formation of the more distal elements but these were atypical. These graft wings were very similar to the legs in which the apical ectodermal ridge had persisted for a longer time than is usual for the wings.

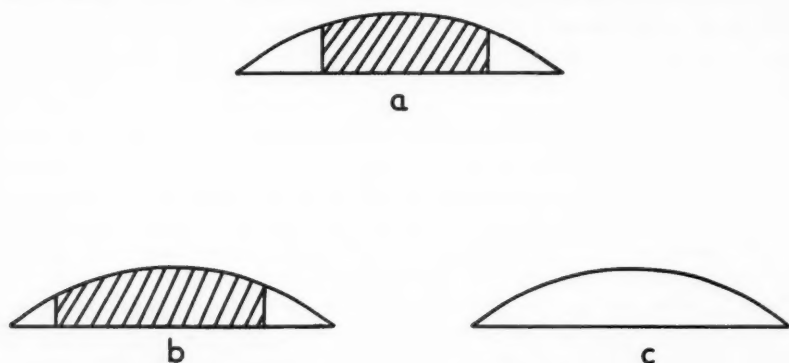


FIGURE 2. Diagram to show distribution of the ectodermal ridge maintenance factor in the mesoderm of a) normal limb bud, b) pre-axial polydactylous limb bud and c) wingless limb bud.

Evidently one of two things is happening in the wingless homozygotes. Either something detrimental to the continued existence of the apical ectoderm is produced by the limb mesoderm or something necessary for the continued existence of Saunders' ridge is missing. There is, at the present, no experimental basis for a choice between these alternatives. However, if one adopts the latter possibility one may have a reasonable hypothesis which accounts for all of the data so far obtained in experiments with limb buds. The hypothesis is this: outgrowth of the limb mesoderm and the formation of distal structures is dependent on some inductive stimulus which comes from the apical ectodermal ridge. The apical ridge is, however, not independent and self-sustaining. It depends upon some factor normally present in the mesoderm for its continued existence as an active (thick) structure. This can, at the present, be regarded merely as a maintenance factor. Moreover this maintenance factor for the apical ridge is not distributed uniformly through the limb bud mesoderm. More of it is present in the central region of the limb bud and less at both ends (Fig. 2a). If this were not so and if the apical ridge were equally active in stimulating outgrowth throughout its length then limbs would be round burgeoning struc-

tures. According to this hypothesis the results with both of our genetic deviants can be explained. Polydactylous conditions result when the maintenance factor is distributed farther preaxially than in normal limb buds (Fig. 2b). This produces a more extensive apical ridge and consequently more preaxial outgrowth. Winglessness is a consequence of the absence of the maintenance factor (Fig. 2c). The ectodermal ridge forms, but cannot persist because of this deficiency. Ergo—no outgrowth. When the maintenance factor is present in minimal amounts, as in the legs of homozygotes, distal development may continue as long as the factor lasts, but eventually the apical ridge regresses and distal outgrowth stops.

All of the limb asymmetries, which were described earlier, may be accounted for by an asymmetrical distribution of the maintenance factor. The regions of high activity of the ridge would then correspond with the regions of the mesoderm in which there are high concentrations of the maintenance factor. Supporting evidence for this view comes from some of our experiments with normal limb buds.

The combination of wing bud mesoderm and leg bud ectoderm developed into a perfect wing. The reciprocal combination yielded a normal leg (Zwilling, 1955). The apical thickening conformed to the pattern of distribution of the maintenance factor in the mesoderm. When ectoderm was rotated 180° before it was placed upon the mesoderm from an homologous bud the ectodermal ridge again conformed to the mesoderm and all of the axial relations of the limb which developed were those expected of the mesoderm (Zwilling, 1956c). In another experiment the thick, most active regions from two or three apical ectodermal ridges were placed in tandem along the apical edge of the mesoderm from one limb bud. These regulated so that the distribution of thin and thick ridge was normal in a short time and normal limbs developed (Zwilling, 1956a).

We may return now to the introduction and compare the chick limb buds with those of amphibia. Is the mesoderm of the chick limb bud "the positive factor of limb formation"? It contains the factor necessary for the existence of the ectodermal ridge and thereby also controls the pattern of development. These are certainly important positive attributes. But none of these traits can be expressed unless the proper kind of limb ectoderm is associated with the mesoderm. Unlike the situation in the amphibia, other ectoderm cannot, at the stages tested, take the place of the apical ectodermal ridge nor can the latter regenerate when it is completely excised. Therefore, it too, is an important "positive factor" for limb development. The nature of the relationship of the two "positive factors" for limb development is such that one cannot express its morphogenetic potentialities without the other—there is a strong reciprocal dependence between them. We have performed an experiment which indicates that the nature of this reciprocal relation may be altered and that the ectoderm may control one of the "positive" features usually resident in the mesoderm. As indicated above, when the ectoderm was rotated 180° the axial relationships which developed were those of the mesoderm. However, when the ectoderm was

rotated 90° in similar experiments all of the axial relationships were determined by the ectoderm (Zwilling, 1956c). In assessing capacities of the components of systems such as this, one must be careful to evaluate them under conditions of greater and lesser stability. Unsuspected properties may be revealed when the system is unstabilized. Future experiments may show that the ectoderm is capable of controlling even more of the "positive factors" of limb development under other altered conditions.

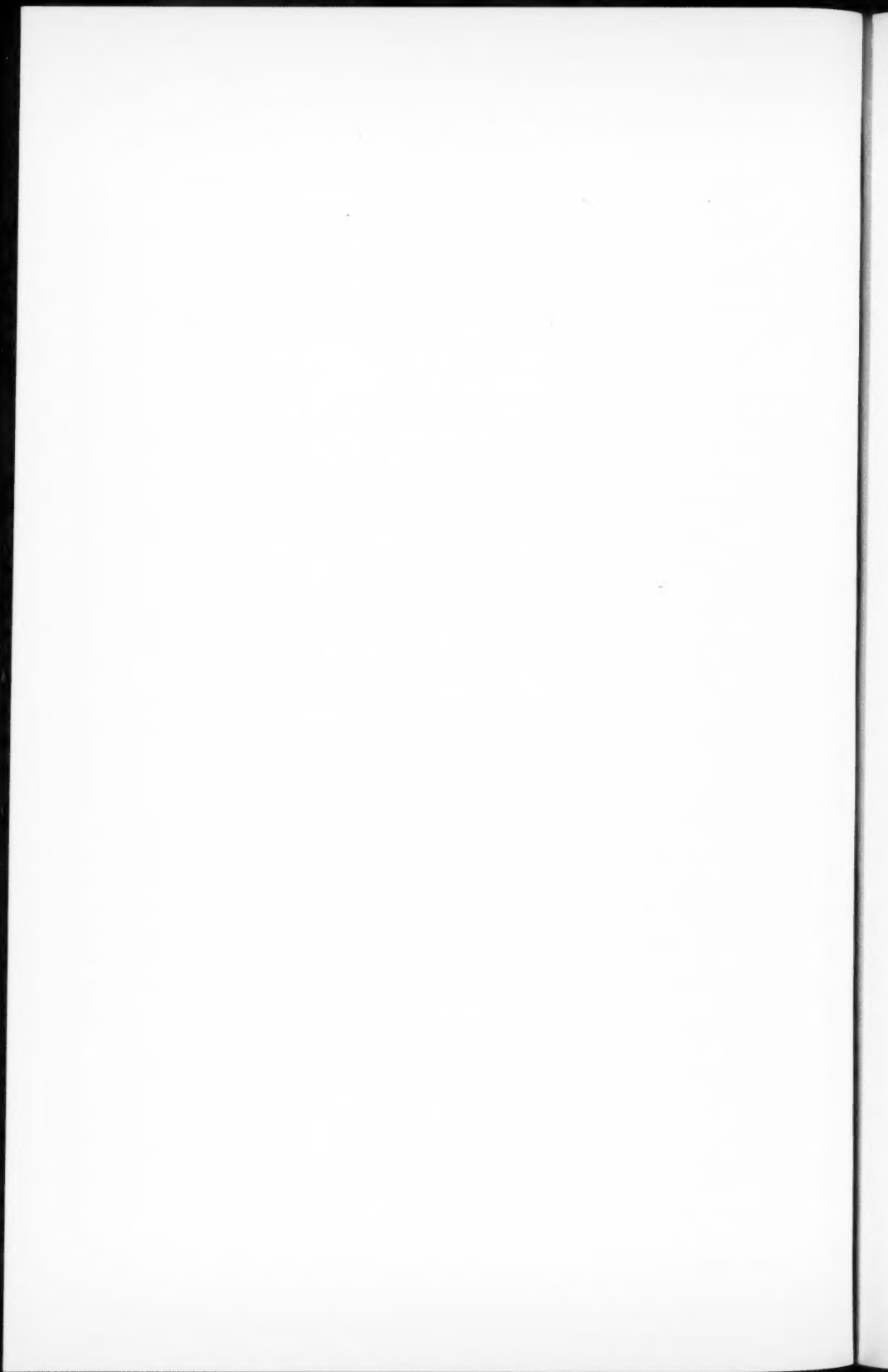
SUMMARY

An hypothesis is presented which postulates that limb bud ectoderm and mesoderm have a reciprocal dependence. According to this view the chief function of the limb bud's apical ectodermal ridge is to promote continued outgrowth of the mesoderm. In addition to responding to this stimulus the mesoderm contains a factor for continued persistence of the ectodermal ridge. Asymmetries of the limb develop because of the asymmetrical distribution of the maintenance factor in the mesoderm. This determines which regions of the ectodermal ridge will become thick and active and thereby controls many aspects of limb pattern. The action of two mutations which have been studied seems to be primarily upon the maintenance factor. The distribution is altered in polydactylous limbs while the wing buds of a "wingless" mutant appear to be deficient in the maintenance factor.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A DESCRIPTION OF A RED DRUM, *SCIAENOPS OCELLATA*, WITHOUT EYES, WITH A CONSIDERATION OF THE IMPLICATIONS

The red drum or redfish, as it is called on the Gulf Coast of the United States, is possibly more prone to the development of certain anatomical abnormalities than other fishes. I described two specimens from Texas with reversed scales (Gunter, 1941, 1945a). I also listed a specimen without eyes (Gunter, 1945b), on the basis of a report of a commercial fisherman. This faith has been verified finally by the acquisition of a second specimen without eyes.

The specimen was caught in Alazan Bay on the south Texas coast some two to four days before it was brought to Mr. H. C. Ware, of Riviera, Texas, on June 21, 1955. The collector and the method of capture are unknown to me. Mr. Ware gave the fish to the Marine Biology Laboratory of the Texas Game and Fish Commission at Rockport, and I am deeply indebted to Mr. Howard Lee and Mr. Cecil Reid of that laboratory, who, knowing my interest in the specimen, generously presented it to me.

The fish was 63.5 cm. (25 in.) in fork length and 68.6 cm. in total length. It had been gutted and gilled and weighed seven pounds in that state. It was a male and the testes were fairly well-developed. The animal would have spawned in the fall. It was in good condition and there was a considerable amount of fat on it. After the preliminary examinations and the photographs were made, the body was eaten because it appeared to be perfectly normal. The head was preserved and is in my possession. Anatomical studies will be made later in comparison with a normal redfish, and the results will be presented elsewhere.

Plate I shows that the fish had no eyes at all and not even vestigial eyes are apparent from the external examination. It is doubtful that the fish was light-sensitive.

DISCUSSION

The redfish belongs to the Sciaenidae or drum family, which is in many ways the most successful family of fishes on the northern Gulf coast (see Gunter, 1938). It is a fast, strong, active fish, highly valued by both sportsmen and commercial fishermen alike. It is a predatory species feeding largely on shrimp and crabs and is not a bottom feeder like its relative, the black drum, *Pogonias cromis*, which subsists largely on molluscs.



PLATE I-1a. A head view of the eyeless redfish.

The black drum has sensory barbels under the chin; the redfish does not. Yet the redfish can change its habits to such an extent that it can exist perfectly well, so far as bodily condition indicates, in a completely blind condition.

Mr. Samuel Gampert, a long-time fisherman at Port Aransas, Texas, told me that he once caught a blind redfish in a cast-net. It had opaque eyes and presumably had come from the Laguna Madre, where the high salinity sometimes causes fishes to go blind. He released it and it moved away, not with the headlong rush of a normal redfish under such circumstances, but very slowly.

The first blind redfish reported (Gunter, 1945b) was sold on the open market and it must have been in good condition. The flesh of the specimen reported here was in excellent condition. Blind redfish must have different feeding habits from normal individuals, but how different is not known, and they may, conceivably, not be greatly different for redfish sometimes feed on the darkest nights when nothing can be seen.

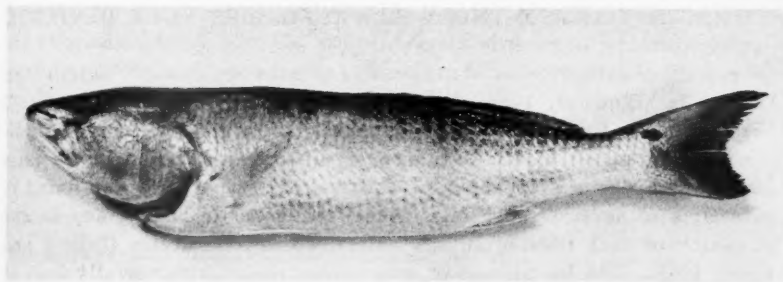


Plate I-1b. A view of the left side of the whole fish.

Redfish with intermediate development of the eyes are not known. A few may be hatched without eyes, but unfortunately there is no surety about that question. Pew (1955), who gave a popular account of this specimen, has pointed out that the eyeless condition could be the result of a congenital anomaly, a pathological anomaly, heredity, or physical removal of the eyes by parasites or predators. It should also be noted that the specimen came out of the Laguna Madre, where the hypersaline condition sometimes causes redfish and other fishes to go blind. Possibly the high salinity could cause the eyes of a small redfish to degenerate. Unfortunately, it is doubtful that the question can be answered by anatomical dissection.

It is generally assumed that the evolutionary degeneration of the eyes of certain cave animals is slow. The existence of successful blind redfish indicates that this is not necessarily true, at least from the ecological standpoint.

If such eyelessness in redfish is hereditary, then in the cases reported, in time and space, two "hopeful monsters" almost met. The same can be said of the two fish with reversed scales. However, it is scarcely conceivable that such progeny could compete successfully with their better endowed congeners.

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MELANOTIC TUMORS IN DROSOPHILA AS POSSIBLE PHENODEVIANTS

Several investigators of the so-called benign melanotic "tumors" in *Drosophila* (Burdette, 1954; Wilson, King and Lowry, 1955, and others) have established that the appearance of these tumors is genetically conditioned. Such tumors appear spontaneously in scattered individuals in most laboratory stocks of *Drosophila melanogaster*; they have also been found in wild flies of several *Drosophila* species collected in nature, and in the progenies of such flies in strains maintained in laboratories (Brncic and Koref, 1953). The frequencies of spontaneous tumors are generally low, of the order of 1 per cent or lower. Makino and Kanehisa (1951) showed however, that these frequencies can be substantially increased by inbreeding, although seldom to 100 per cent. The morphology, localization and number of the tumors in the body of the larva or adult fly are characteristic for different stocks.

The genetic studies of Burdette and other authors have been interpreted to mean that the tumors are produced by one, two, or more specific genes in different stocks. These genes are always incompletely penetrant, and vary in expressivity, sometimes due to systems of modifiers. The main genes and modifiers responsible for the production of tumors have been shown to be localized in all chromosomes, except the small fourth in *Drosophila melanogaster*. However, these genes in different strains are, with one known exception, not allelic (Burdette and Olivier, 1952). The increase of tumors is likewise greatly influenced by environmental variations. (Burdette, 1954; Wilson, King and Lowry, 1955; Brncic and Koref, 1951, 1953).

Our recent studies lead us to believe that the appearance of melanotic tumors in *Drosophila* may best be interpreted in the light of the recent work of Lerner (1954) and others. These tumors may be what Lerner has called "phenodeviants." The incidence of tumors seems to depend not so much on individual mutant genes as on the general genetic environment, the residual genotype, of the strains in which they are found. Table 1 summarizes some relevant data. Three strains of *Drosophila melanogaster*: tu^{48j} , tu^{49h} , and $tu\ vg\ bw$, have been used. The incidence of adult flies in these strains showing one or more melanotic tumors is 48.3, 63.2, and 99.9 per cent, respectively. Each of these strains was outcrossed to the wild Oregon R-c strain and to seven different mutant strains listed in the table. In the F_1 generation of these crosses no tumors at all have been found in the 200 flies examined from each cross (a total of 4800 flies). The tumors have reappeared, however, in the F_2 hybrids. The table shows that the frequencies of the affected flies were very different in different crosses, although always lower than in the original tumor stocks. The crosses to the Oregon R-c strain have been taken as the standard; it is known that tumors in this strain are rare (about 0.1 to 0.2 per cent). The column marked "Sig. Dif."

in the table shows the differences between the cross to Oregon R-c and to other strains, divided by the standard errors of these differences. In several cases the ratios are higher than three, and therefore indicate statistically significant deviations.

TABLE I
INCIDENCE (IN PER CENT) OF TUMORS FOUND IN F2 IN CROSSES
BETWEEN TUMORAL AND NON TUMORAL STOCKS.

Non tumoral stocks	tu 48j			tu 49h			tu vg bw		
	No. of indiv.	Per cent of tu	sig. Dif.	No. of indiv.	Per cent of tu	Sig. Dif.	No. of indiv.	Per cent of tu	Sig. Dif.
Oregon R-c	2085	0.48	2122	2.81	2254	3.54
w	2034	0.35	0.65	1667	2.84	0.05	2213	2.45	2.01
f	1566	0.32	0.77	1314	2.89	0.13	1914	1.00	5.64
dp	2116	1.4	3.2	1760	3.53	1.28	1667	16.73	13.32
bw	2016	0.50	0.09	2262	8.44	8.13	2179	5.26	2.81
st	2076	0.48	0.00	2196	5.07	3.81	2119	2.81	1.004
se	714	8.02	7.39	2750	29.17	28.34	1838	12.13	10.105
ey	2042	0.54	0.02	2081	3.02	0.41	2198	4.86	2.2

We may, then, conclude that: (1) Tumors appear in most species and strains of *Drosophila*. (2) They are produced by different "principal genes" in different strains, most of them non-allelic. (3) The penetrance is characteristic for each strain, and depends on the residual genotype of this strain. (4) The frequency is increased by inbreeding and decreased by outbreeding. (5) The frequency is sensitive to environmental influences. All these characteristics of the "tumors" resemble those of traits such as "podoptera" in *Drosophila* (Goldschmidt, Hannah and Piternick, 1951), and as "crooked toes" in chickens (Lerner, 1954). These traits are considered by Lerner as "abnormal morphological deviants (phenodeviants) which occur sporadically, caused by the intrinsic properties of Mendelian inheritance, due to which a certain percentage of individuals of every generation falls below the threshold of the obligate proportion of loci needed in heterozygous state to insure normal development."

It may be interesting to consider the possibility that susceptibility for the production of tumors in *Drosophila*, and perhaps in other animals, may not be caused by mutations at specific loci. Their origin may rather be due to loss of the normal genetic equilibrium, or of genetic homeostasis in certain strains. This loss may be produced in particular by inbreeding, connected or unconnected with selection. If so, the production of tumors is due not to genes specifically concerned with tumors, but to alleles of loci which exist in most normal individuals of a species or a population.

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